Cardio vascular disease in rheumatoid arthritis: risk profile and risk prediction

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ISBN
978-94-91602-90-0

Cover design
Eugene Arts

Layout
Elke Arts

Print
Print Service Ede

The publication of this thesis was financially supported by Pfizer bv.

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Proefschrift
ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. dr. J.H.J.M. van Krieken,
volgens besluit van het college van decanen
in het openbaar te verdedigen op vrijdag 31 maart 2017
om 12.30 uur precies

door

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Geboren op 26 juni 1986
te Nijmegen

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CHAPTER 1

GENERAL INTRODUCTION

In the Saltpêtrière asylum in France, physician Augustin Jacob Landré-Beauvais first noticed and described the symptoms and signs of patients with severe joint pain that could not be explained by other conditions of the joints known at that time (1820s). This condition affected mainly the poor, women more often than men and it was first described as rheumatoid arthritis (RA) by Archibald Garrod in 1890.[1] Its etiology was unclear, and many theories have been proposed since then. Today, the exact cause of RA remains unknown. However, extensive research has generated much knowledge about its presentation and diagnosis, the pathophysiology, disease course, prognosis and treatment.[2-6] RA is an auto-immune disease that is characterized by chronic systemic inflammation that mainly affects the small joints of the hands and feet, leading to pain and stiffness, fatigue and disability, and largely irreversible joint damage if left untreated. The prognosis for patients with RA has improved significantly during the last two decades. This achievement can be primarily attributed to earlier diagnosis, and treatment targeted to low disease activity or remission by using (combinations of) disease-modifying anti-rheumatic drugs (DMARDs) including biological DMARDs.[7, 8]

Cardiovascular disease in RA

Despite these advances, cardiovascular morbidity and mortality rates in patients with RA are increased compared to the general population.[7, 9-11] Extensive research has shown that cardiovascular disease (CVD) accounts for a substantial part of the excess mortality found in RA.[12-16] Reported age and gender adjusted standardized mortality ratios for cardiovascular death are approximately 1.3-1.6 times higher than in the general population.[13, 17-19] A study by Peters et al. found the risk to be comparable to the risk of CVD reported in patients with type 2 diabetes mellitus.[20] Illustratively, the incidence of myocardial infarction (MI) in RA patients was found to be significantly higher compared to age and gender matched controls.[15, 21] In a prospective cohort study that included 114342 women, with 2.4 million years of follow-up, the risk of an acute MI was found to be two times higher in women with RA compared to women without RA.[22] It has also been shown that an increased risk of CVD is present even in preclinical stages and in very early RA.[23-25] RA patients are more likely to develop atherosclerotic plaques, silent ischemic disease and they have an increased risk of sudden cardiac death, compared to the general population.[13, 25] In order to decrease CVD morbidity and mortality in RA, tangible risk factors that can be targeted for the prevention of CVD need to be identified. Consequently, it is necessary to gain detailed knowledge on mechanisms that cause the excess CVD risk and the predictive power of appointed risk factors in this population.

Risk factors for cardiovascular disease in RA

In the general population, CVD risk management is focused on identifying and treating risk factors included in the CVD risk profile such as smoking, hypertension, diabetes mellitus, high total cholesterol (TC) levels, high levels of low-density lipoprotein cholesterol (LDL-c), or low levels of the protective high-density lipoprotein cholesterol (HDL-c).[26, 27] It has been reported that these “traditional” risk factors may have a different effect on CVD risk in RA patients compared to the general population.[28-30] Also, the prevalence of certain risk factors such as smoking, insulin resistance, sedentary lifestyle and hypertension was shown to be increased in RA.[31-35]

At the same time, there is evidence that suggests that RA patients are undertreated for CVD risk.[36, 37]. Interestingly, it has become evident that traditional risk factors do not fully account for the excess risk of CVD in RA.[13, 22, 28, 38] Therefore, there may be other useful targets that contribute to the development of CVD in RA. It being an auto-immune disease, systemic inflammation and immune mechanisms have been suggested as important disease-related CVD risk factors in RA.[39, 40] This is particularly supported by the fact that atherosclerosis, which has been described as an inflammatory disease, appears to be accelerated in RA.[40-43] In addition, C-reactive protein (CRP), an inflammatory marker used to determine disease activity in RA, is predictive for atherosclerosis.[44, 45] Indeed, both acute phase reactants CRP and erythrocyte sedimentation rate (ESR), have been associated with CVD in RA and polyarthritis.[9, 46-49] Moreover, atherosclerotic plaques appear to be more severe and prevalent in RA patients in comparison to the general population.[42, 50-53] These findings have been reported in both early and established RA.[42] Also, in comparison to healthy controls and RA patients in remission, RA patients with active disease have an increased risk of unstable plaques that are more prone to rupture and cause CVD.[54] Inflammation also appears to modify the effect of traditional risk factors, particularly lipoproteins.[55-61] Notably, the anti-atherogenic properties of HDL-c appear to be diminished as a consequence of chronic inflammation, even becoming pro-inflammatory.[58-61] This may in part be due to altered composition of HDL-c particles, however this has not been investigated in RA patients. In addition to determinants of inflammatory activity, other 'disease-related' markers have been identified as potential novel risk factors for CVD in RA, notably rheumatoid factor (RF) positivity and anti-citrullinated protein antibody (ACPA) positivity.[19, 47, 62]

Disease activity as risk factor for CVD in RA

In RA patients, inflammatory activity can be quantified by disease activity measures such as the 28-joint disease activity score (DAS28). The DAS28 is a combined index that consists of four components; number of swollen joints, number of tender joints, an inflammatory biomarker (either CRP or ESR) and the general health score (Visual Analogue scale [VAS]). Twenty-eight joints are scored and disease activity is estimated by entering the found results into the DAS28-formula.[63] There is conflicting evidence about the association between disease activity and CVD events. Using the DAS28, Radovits et al. reported that disease activity overall was not significantly associated with the occurrence of MI in a case-control study.[64] On the other hand it was shown that exposure to frequent flare ups of disease activity and increased burden of RA disease activity over time increases the risk of CVD.[65, 66] Solomon et al. demonstrated that reduced time-averaged disease activity was associated with fewer CV events in a cohort of RA patients with a median follow-up of 2.7 years.[65] Furthermore, a meta-analysis investigating the association between anti-rheumatic drugs and CVD in RA and psoriatic arthritis reported a reduced risk of CVD in patients treated with methotrexate or TNF-alpha blocking agents. Whether this reduction is (partially) achieved through a reduction in disease activity has not been fully elucidated.[67] Conversely, the absence of clinical disease activity (i.e. remission) may protect against excess CVD risk. However, this hypothesized association also requires further investigation as it is unclear if low disease activity would be sufficient, or if absolute remission is required. In addition to disease

activity, disease duration could be a factor that contributes to the risk of CVD. Studies investigating the effect of disease activity on CVD risk in RA are likely to be limited by small patient samples, a relatively short follow- up, lack of data on traditional CVD risk factors in RA cohorts or cross-sectional designs not quite suited to investigate such relationships or long-term outcomes such as CVD. Furthermore, heterogeneity of patient samples, fluctuating disease activity over time and the large variety of applied treatment strategies between individual patients further complicates most studies. Overall the relationship between clinically measured disease activity and CVD has not been sufficiently clarified. Additional research is necessary to determine whether suppression of the inflammatory process with effective treatments would have a lasting beneficial effect on the risk of CVD.

The interplay between traditional and disease related CVD risk factors

The discovery of disease-related CVD risk factors eventually led to the 'smaller slice of a larger pie' hypothesis (Fig.1.1).[68] RA patients have an increased risk of developing CVD and traditional risk factors are certainly of importance in that respect. However, it is likely that they account for a smaller proportion of total risk of CVD in RA compared to the general population. The CVD risk profile in RA appears to consist of a complex interplay of pathophysiological mechanisms that influence each other and contribute to the risk of CVD in RA. As discussed in the previous paragraphs, inflammation may modify the effect of traditional CVD risk factors, as well as act as a CVD risk factor in its own right. Consequently, risk prediction in individual RA patients using only the traditional risk factors as they are defined in the general population would probably lead to suboptimal results.

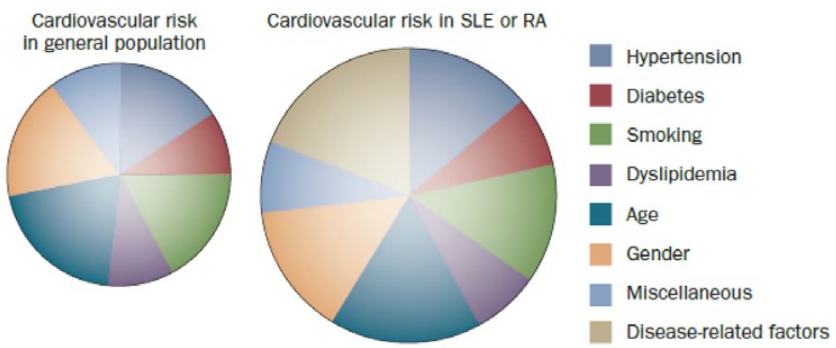

Figure 1.1. Hypothetical distribution of cardiovascular risk factors in patients with systemic lupus erythematosus or rheumatoid arthritis in comparison with the general population. Risk of cardiovascular disease is increased in these two patient populations, however compared to the general population the relative contribution of traditional cardiovascular risk factors is smaller due to competing risks caused by the presence of inflammatory rheumatic disease. Reprinted from Epidemiology of CVD in rheumatic disease, with a focus on RA and SLE, by DP. Symmons and Gabriel SE, 2011, Nat Rev Rheumatol,7, p387. Copyright 2016 Macmillan Publishers Limited, part of Springer Nature. Reprinted with permission.

Risk prediction in Rheumatoid Arthritis

Risk prediction is widely applied in the field of CVD. It is the process in which information that is available now is transformed into a probability that estimates the actual state of the subject at some point in the future. When risk prediction research is performed properly it can contribute to the identification of suitable (new) risk factors and potential therapeutic targets.[69] Risk prediction in individuals can be used for primary or secondary prevention and risk estimates can also be used to raise awareness of CVD within a target population.[69] Furthermore, in clinical practice it can be a useful tool to communicate the message of an increased risk of a future outcome, motivating patients to adhere to lifestyle adjustments and/or drug therapy. In the field of CVD, decades of research have produced a certain set of predictors that have proven to be strongly associated with the risk of future CVD and are therefore included in most current risk CVD risk algorithms.[70-72] Many of these traditional risk factors for CVD were identified in the Framingham Heart Study that was initiated in 1948. This US research group also developed one of the first CVD risk algorithms that could be used in clinical practice; the Framingham risk score.[73] A European counterpart was developed; the Systematic Coronary Risk evaluation score (SCORE).[70] The FRS and SCORE algorithms were adapted to predict CVD morbidity as well as CVD mortality.[71, 74] However, the FRS, SCORE and other CVD risk models were not validated for use in the RA population. This may be of importance as these risk algorithms mainly include traditional risk factors and do not account for the effect of inflammation on these risk factors. More recently developed risk models that include rather crude measures of inflammatory activity such as the Reynolds Risk Score that includes CRP [72, 75] or the QRISK II score [76] that includes diagnosis of RA as a risk factor have not been validated in RA patients either.

In conclusion, there is a growing body of evidence that not only supports the notion that CVD substantially contributes to premature mortality in RA patients, but also leads to the hypothesis that the CVD risk profile of RA patients significantly differs from the general population, questioning the validity of existing risk management strategies for the prevention of CVD. There is a need for evidence based guidelines for the management of CVD risk in RA patients that specify targets for CVD prevention and risk estimation in this population.

Outline thesis

Overall the purpose of this thesis is to further support the formation of evidence based recommendations for CVD risk management strategies in the RA population. The main focus of this thesis will be on the analysis of CVD risk prediction in RA and on contributing to the definition of the CVD risk profile in RA patients. More specifically, in this thesis, the performance of CVD risk prediction models is evaluated in RA patients and the added value of RA-related risk factors as predictors of CVD risk are investigated. This thesis aims to shed more light on the relationship between disease activity and CVD. To investigate CVD in RA, long-term follow-up is often required as it generally takes years to develop CVD. Also, data on CVD risk factors, comorbidity and detailed follow-up data that includes disease activity is needed. In most existing RA cohorts, data on traditional CVD risk factors are not routinely acquired. For this thesis, almost all of the necessary data could be extracted from the Nijmegen inception cohort database,[77] with the exception of

lipid levels that were not determined at baseline. Instead, stored serum samples were available for the determination of cholesterol levels, but the integrity of these samples may be affected after frozen storage. Literature regarding this issue is limited, particularly when it involves long-term storage. Therefore, in **Chapter 2**, the effect of long-term storage on the validity of cholesterol measurements using stored serum samples is investigated. In **Chapter 3**, the predictive performance of four current risk algorithms (FRS, SCORE, Reynolds risk score, Q-Risk II) used for the estimation of the 10-year risk of CVD in the general population is evaluated. These risk models form the basis of widely propagated CVD risk management guidelines used in both the US and Europe. However, they have not yet been validated in an RA population. Next, disease specific risk factors are investigated in order to further define the CVD risk profile of RA patients, focusing particularly on the effects of inflammation on the risk of CVD. Inflammation appears to affect lipoprotein patterns found in RA patients. Both the concentration and function of certain lipoproteins appear to be modulated under the influence of inflammation.[57, 60, 78-80] In **Chapter 4**, the effect of inflammation on the composition of HDL-c is determined in patients with RA. Evidence suggests that chronic inflammation may lower HDL-c levels.[57, 79] Also, inflammation may alter or even diminish the beneficial, anti-atherogenic properties of HDL-c,[58-60] possibly by affecting HDL composition. Furthermore, TC and HDL-c are of particular interest as the TC:HDL-c ratio or atherogenic index (AI) is used in most CVD risk algorithms. The AI is the ratio of the "bad" cholesterol (TC) versus the "good" cholesterol (HDL-c). The concentrations of these two types of cholesterol seem to rise and fall in the same direction, subsequent to fluctuations in disease activity during the course of RA.[81, 82] This phenomenon has led to the hypothesis that the AI is less susceptible to changes in disease activity. To clarify this issue, a systemic review of the available literature was performed, focusing on the relationship between therapy with biological disease modifying anti-rheumatic drugs (DMARDs) and its effect on the AI. This review is presented in **Chapter 5**. Disease activity has been suggested as an important disease specific risk factor for CVD in RA. Particularly patients with poorly controlled disease over time are exposed to long-term systemic inflammatory activity which could increase CVD risk in these patients. However, this hypothesis is primarily based on research focusing on the effect of inflammation on atherosclerosis. Also, the effect of disease duration on the risk of CVD during long-term follow-up requires further clarification. Therefore, the effect of disease activity and disease duration on the risk of "hard" CVD outcomes was investigated in **Chapter 6**. Effective anti-rheumatic treatment seems to reduce CVD morbidity and mortality.[67, 83-85] Treatment strategies aimed at significant reduction or even complete (clinical) eradication of the inflammatory auto-immune response (i.e. remission) in RA may reduce CVD risk even further. In **Chapter 7**, the effect of low disease activity and remission over time on the risk of CVD was investigated. Finally, this thesis focuses on the development of a CVD risk model specifically suited for use in RA patients. In **Chapter 8**, the SCORE risk model was recalibrated and adapted for use in the RA population, integrating disease specific risk factors into the model. The predictive performance of this adaptation was evaluated and compared to the original SCORE algorithm. A comprehensive discussion of our findings and recommendations for future research are provided in the general discussion in **Chapter 9**. A summary of all results and the conclusions of this thesis are presented in **Chapter 10 & 11**.

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CHAPTER 2

Serum samples that have been stored long-term (>10 years) are a suitable data source for developing cardiovascular risk prediction models in large observational rheumatoid arthritis cohorts

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BioMed Research International
2014; doi 10.1155/2014/930925

ABSTRACT

Objective

There is an unmet need for a specific cardiovascular risk (CV) algorithm for rheumatoid arthritis (RA) patients. Often, data on lipoproteins are not available in RA cohorts but could be obtained from frozen blood samples. The objective of this study was to estimate the storage effect on lipoproteins in long-term (>10 years) frozen serum samples.

Methods

Data were used from an inception RA cohort. Multiple serum samples from 152 patients were analyzed for lipoproteins, being frozen for 1–26 years at −20◦ C. Storage effect on lipoproteins was estimated using longitudinal regression analyses and a lipid decay correction factor was developed. Clinical impact of the storage effect on lipoproteins was assessed by calculating the number of patients reclassified to another CV risk group according to the SCORE risk calculator after applying the decay correction factor.

Results

There was a significant effect of storage time on total cholesterol (TC) ($p<0.001$) and high density lipoprotein cholesterol (HDL-c) levels ($p<0.001$), not LDL-c ($p=0.83$). The lipid decay correction factor was 0.03 mmol/L and 0.02 mmol/L per additional year of storage for TC and HDL-c, respectively. The TC:HDL ratio decreased after correction for storage effect. After correction, 5% of patients were reclassified to another CV risk group.

Conclusion

A modest storage decay effect on lipoproteins was found that is unlikely to significantly affect CV risk stratification. Serum samples that have been stored long-term (>10 years) can be used to obtain valid lipid levels for developing CV risk prediction models in RA cohorts, even without applying a decay correction factor.

INTRODUCTION

Cardiovascular risk is increased in patients with rheumatoid arthritis (RA).[1] Cardiovascular disease (CVD) accounts for 50% of all excess mortality in RA patients.[1] RA itself as a chronic inflammatory condition may increase CVD risk. Also, studies have shown that inflammation may modulate traditional CVD risk factors.[2, 3] Atherosclerotic plaques in the carotid artery appear more severe and prevalent in RA patients compared to the general population.[4–7] In comparison to healthy controls and RA patients in remission, RA patients with active disease seem to have less stable plaques that are more vulnerable to rupture, which increases the probability of a cardiovascular event. Considering the increased risk, prevention of CVD is important. According to international guidelines this includes adequate risk assessment using a CVD risk algorithm. Several risk algorithms are available in the general population, such as the systematic coronary risk evaluation (SCORE) and the Framingham risk score [9, 10] which were not validated in RA patients. As traditional risk factors, upon which these models are based, do not fully account for the excess CVD risk, their predictive performance may be suboptimal. It has recently been reported that both the SCORE and Framingham risk score algorithm provide suboptimal CVD risk estimates in patients with RA.[11, 12] To improve CVD risk assessment in RA patients, disease specific risk factors may be required such as RA disease activity. Also, other cardiovascular-related parameters not incorporated in the present algorithms, such as carotid artery intima-media index (cIMT), the presence of plaques in these patients, or certain genetic markers associated with CVD risk factors, might also be taken into consideration.[13–17] There is an unmet need for a RA specific CVD risk calculator. In order to evaluate current risk models and develop and validate an RA specific CVD risk model, it would be advantageous to use data from existing cohorts with long follow-up. In existing cohorts of RA patients, lipid levels are often not determined at baseline. Long-term storage may lead to degradation of cholesterol, that is, a lipid decay effect. Consequently, if lipids were to be measured in samples that have been stored for longer periods of time, cholesterol levels could be underestimated. Furthermore, if these measurements were used in CVD risk algorithms, the result may be an underestimation of CVD risk. Within a timeframe of 1-2 years of storage, no change to moderate decreases in lipid levels have been reported,[18–20] as well an increase of high density lipoprotein cholesterol (HDL-c) levels.[21, 22] Lipid decay seems to be smaller at lower temperatures.[18–20] This has led to the hypothesis that the HDL-c concentration influences the effect of storage on lipoproteins.[23] Overall, the storage decay effect in total cholesterol (TC) and triglyceride (TG) levels seems smaller when stored than in HDL-c levels.[24, 25] One study has investigated the effect of long-term storage on cholesterol levels for up to seven years of storage. A significant mean decrease of 2.0% per year storage in TC levels and a nonsignificant average 1.3% decrease per year storage in HDL-c levels were reported.[26] To our knowledge, the effect of longer storage (>10 years) on serum cholesterol levels has not been investigated. Although deterioration of cholesterol content in stored serum samples can be expected, the magnitude of this effect after long periods of time is unknown. The objective of this study is to estimate the long-term storage decay effect on TC and HDL-c levels in frozen serum samples of RA patients and to evaluate the clinical effect of the decay in a CVD risk model.

METHODS

Study Design

Serum samples taken at baseline and at 1, 2, 3, 5, 7, and 10 years of follow-up from patients included in the RA inception cohort of the Radboud University Nijmegen Medical Centre, from 1985 up to 2009 (n = 640), were used for measurements of lipoproteins. To test for a period effect, patients were stratified in five subcohorts according to year of inclusion in the cohort during 1985–1989, 1990–1994, 1995–1999, 2000–2004, and 2005–2009 (Figure 1). The study was approved by the Medical Ethical Committee and CMO Arnhem Nijmegen and informed consent was acquired from all participants.

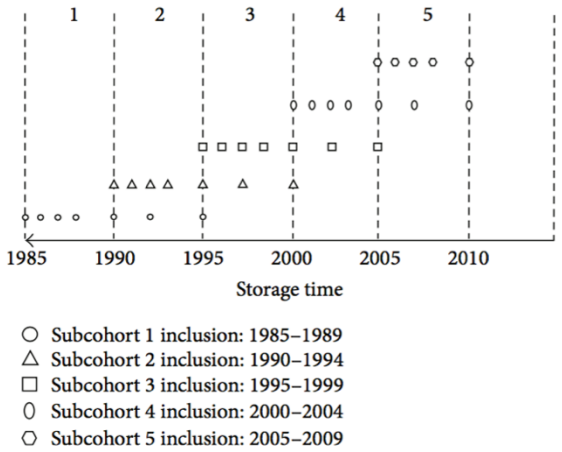

Figure 1. Storage and blood sampling times for the 5 subcohorts. Symbols represent the time points of the included selection of stored serum, during follow-up (baseline and year 1, 2, 3, 5, 7 and 10).

Patients

Inclusion criteria for the early RA cohort were; fulfillment of the 1987 ACR classification criteria for RA, disease duration <1 year, and being DMARD (disease-modifying antirheumatic drug) naive. From this cohort, we selected at random 150 RA patients from the inception cohort using computer generated random numbers, to obtain 30 samples per subcohort.

Serum Samples

During follow-up, nonfasting blood samples were drawn annually by a trained nurse. Approximately 400 mL of serum was stored from each sample and divided into four separate vials. The samples were initially stored at −20◦ C. In 2008, all samples were transferred to storage facilities at −80◦ C. Blood samples collected from 2008 and thereafter were stored directly at −80◦ C. Blood samples obtained before 2007 were stored in 1.5 mL Eppendorf vials and samples obtained after 2007 were stored using Greiner “pp Cryovials.” Serum samples that were taken at baseline and during follow-up (at 1, 2, 3, 5, 7, and 10 years) were extracted from storage in January 2012. Immediately following this procedure, samples were prepared for cholesterol

measurements and transported on dry ice to the laboratory facilities of Russells Hall Hospital, Dudley, UK.

Lipid Measurements

TC concentrations were measured enzymatically by means of the VITROS CHOL slide technique using the Triton X-100 surfactant, which is based on methods described previously.[27] HDL-c was measured using immunoturbidimetry. Low-density-lipoprotein cholesterol (LDL-c) was calculated using Friedewald's formula.[28]

Statistical Analysis

The primary outcomes were systematic differences in TC and HDL-c levels measured in the most recently stored samples and measurements from samples that were stored long-term and the secondary outcome was the difference in LDL-c levels. To test for a period effect, a longitudinal regression analysis was used that corrects for repeated measurements within patients. Lipid level (TC and HDL-c) was the dependent variable and follow-up time; subcohort (1985–1989, 1990–1994, etc.) and an interaction term between follow-up time and subcohort were the main independent variables. As the course of cholesterol levels over follow-up time was nonlinear, a quadratic time term (time2) was included. To test for a period effect in the course of cholesterol levels, the interaction between subcohort and follow-up time was evaluated. Several variables were considered as potential confounders: age, gender, statin use at baseline, BMI, smoking, blood pressure, 28-joint disease activity score (DAS28), rheumatoid factor positivity, and glucocorticosteroid use. Variables were considered confounders if their addition to the model led to a >10% change in one of the subcohort follow-up time effects. For the development of a correction factor for the storage decay effect, linear mixed models were used, with cholesterol level as the dependent variable, storage time as primary independent variable, and the same confounders as in the analyses described previously. Storage time was calculated by subtracting the baseline date (date the blood samples were first frozen) from the date of serum analysis. The storage decay correction factor developed to adjust the TC, HDL- c, and LDL-c levels was defined as the estimated change in mmol/L cholesterol (βChol) per additional unit of storage time (years) multiplied by the number of storage years (t) of a particular sample. When added to the measured cholesterol level (βobserved), it gives an estimate of the "original" cholesterol value (y). Therefore, the lipid storage decay factor is: βobserved + (βChol $*$ t). In order to evaluate the clinical effect of the decay in CVD risk models, reclassification across CVD risk groups before and after correction was calculated. The SCORE risk algorithm was used to quantify the 10-year risk of CVD with and without correction for the storage decay effect. The CVD risk was calculated with and without correction for the storage decay effect on lipids for all 1050 patients from the RA inception cohort. Reclassification of patients across CVD risk groups (low <10%, intermediate 10–20%, and high <20%) was calculated. If the estimated CVD risk of a patient exceeds 10%, primary prevention in the form of lifestyle changes or medical treatment is indicated according to European guidelines for CV prevention.[29]

RESULTS

Patients

One hundred and fifty-two patients were included, evenly distributed across the 5 subcohorts (Table 1), with storage times ranging from 1 to 26 years. Samples from the oldest subcohorts comprised the longest storage times. Serum samples from seven time points (0, 1, 2, 3, 5, 7, and 10 years) were analyzed if available, yielding a total of 971 samples. Age, gender, rheumatoid factor (RF) positivity, DAS28, use of statins and glucocorticosteroids appeared to show trends over time (Table 1).

Table 1. Patient characteristics

	Subcohorts				
N=152	1 (N=30) 1985-1989	2 (N=31) 1990-1994	3 (N=30) 1995-1999	4 (N=30) 2000-2004	5 (N=31) 2005-2009
Age (years), mean±SD	51±14.4	50±13.5	52±14.5	58±12.1	59±12.6
Female, N(%)	16 (53.3)	19 (61.3)	17 (56.7)	25 (83.3)	21 (67.7)
RF positive, N(%)	26 (86.7)	23 (74.2)	25 (83.3)	23 (76.7)	25 (80.6)
DAS28, mean±SD	5.6±1.2	5.3±1.4	4.8±1.6	4.9±1.0	5.0±1.2
BMI, mean±SD	26±3.8	27.1±4.6	25.6±3.3	26.2±3.0	26.6±6.8
Smokers, N(%)	14 (46.7)	6 (19.4)	10 (33.3)	9 (30.0)	9 (29.0)
Statin use, N(%)	0 (0.0)	0 (0.0)	2 (6.7)	2 (6.7)	9 (29.0)
Glucocorticosteroid use, N(%)	3 (10.0)	3 (9.7)	5 (16.7)	17 (56.7)	17 (54.8)
Baseline lipoprotein levels					
TC (mmol/L), mean±SD	4.2±1.2	4.5±1.5	5.4±1.0	5.3±1.2	5.2±1.2
HDL-c (mmol/L), mean±SD	0.6±0.2	0.9±0.3	1.0±0.3	1.1±0.2	1.2±0.4
TC:HDL-c, mean±SD	7.2±1.9	5.5±1.6	5.9±1.4	5.1±1.2	4.5±1.2
Treatment during follow-up					
B-DMARDS, N(%)	9 (30.0)	13 (41.9)	10 (33.3	17 (56.7	3 (9.7)

I. Abbreviations: RF, rheumatoid factor; DAS28; 28-joint disease activity score, BMI: body mass index, TC, total cholesterol; HDL-c, high-density-lipoprotein cholesterol, B-DMARDS; biological disease modifying anti-rheumatic drugs

Differences between subcohorts

Lipid levels measured at baseline are presented in Table 1. Lipid levels tended to be lowest in the subcohorts that had the longest storage time (figure 2) and there appeared to be a nonlinear course of lipid levels during storage time. The unadjusted results (not shown) revealed a significant interaction effect between subcohort and follow-up time for TC and LDL-c (p=0.02 and p=0.01, resp.). Overall, the course of the various lipoprotein levels over time was not significantly different between subcohorts after adjustment for confounders (age, gender, and BMI) with p=0.09, p=0.05, and p=0.18 for TC, LDL-c, and HDL-c, respectively. Rheumatoid factor and DAS28 were not confounders after these adjustments. When looking specifically at the oldest and most recent cohort (the two extremes in terms of storage time), lipid levels in the oldest cohort were systematically lower than lipid levers in the most recent subcohort; a statistically significant

difference for TC and LDL-c (p=0.04 and p=0.03) and a nonsignificant difference for HDL-c (p=0.25) were found after correction for confounders.

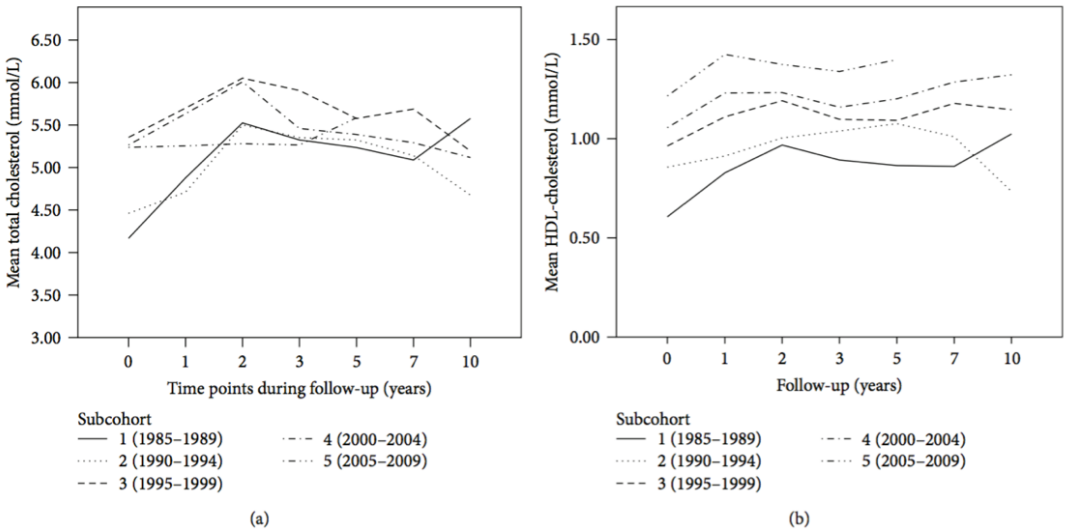

Figure 2. Lipoprotein levels measured in stored serum samples in the various subcohorts. Total cholesterol (a) and HDL-c (b) are depicted on the y-axis. Samples taken at the most recent follow-up moment in time, time point "10" on the x-axis, have the shortest follow-up time and samples taken at baseline (time point "0") have been stored the longest.

Storage time

There was a significant decay effect of storage time on TC and HDL-c levels (p<0.001) (Tables 2 and 3). No effect of storage time was found for LDL-c levels (p=0.83, data not shown). For the analysis for TC and LDL- c, age, gender, BMI, statin use, and glucocorticosteroid use at baseline were confounders and were adjusted for in the analysis. For HDL-c, a model with adjustment for age and gender sufficed. As a significant decay effect was found for TC and HDL-c, a correction factor was estimated. As storage time increased, a decrease (95% CI) was observed, −0.03 mmol/L (−0.045 to 0.015) for TC and −0.024 mmol/L (−0.027 to −0.021) for HDL-c per year of increasing storage time. The decrease in HDL-c levels was relatively larger (considering the range) than the decrease in TC levels at same length of storage. The TC:HDL-c ratio calculated for the same sample will therefore become higher as a direct result of increasing storage time and the disproportionate decay effect on TC and HDL-c. This lipid decay was estimated to be linear. A lipid decay correction factor was calculated to be [y = observed + (Chol ∗ t)]; 0.03 mmol/L for TC and 0.02 mmol/L for HDL-c. Figure 3 illustrates the differences in the course of unadjusted and adjusted cholesterol levels.

Clinical Impact of the Storage Decay Effect on Lipids

The storage decay effect of lipids during storage affects the TC:HDL-c ratio. This ratio is used when calculating the 10-year CVD risk of individual patients in a clinical setting. Patients are then

categorized as either “low” risk (<10% 10-year risk of a CVD event), “intermediate” risk (10–20% 10-year risk of a CVD event), or “high” risk (>20% 10-year risk of a CVD event).

Table 2. Effect of storage time (years) on total cholesterol (TC) levels

	Estimate	SE	P-value	95% CI	
				Lower	Upper
Constant	3.41	0.39	<.001	2.65	4.17
Storage time	−.03	0.01	<.001	−.05	−.02
Time within patients (follow-up)	0.01	0.00	<.001	0.00	0.02
Time^2 (follow-up)	−.00	0.00	<.001	−.00	−.00
Gender	0.02	0.10	0.81	−.17	−.21
Age	0.02	0.04	<.001	0.09	0.02
Statin use at baseline	0.89	0.19	<.001	0.52	1.26
BMI	0.01	0.01	0.34	−.01	0.03
Glucocorticosteroid use at baseline	0.24	0.12	0.04	0.01	0.47

i. SE, standard error; BMI, body mass index; and 95% CI: 95% confidence interval.
ii. Data are adjusted for age, gender, statin use at baseline, BMI, and glucocorticosteroid use at baseline.
iii. Time^2 is a quadratic term that was included due to the nonlinear course of cholesterol levels over follow-up time, and this variable also represents time within patients (follow-up time).

Table 3. Effect of storage time (years) on HDL-c levels, adjusted results.

	Estimate	SE	P-value	95% CI	
				Lower	Upper
Constant	1.25	0.06	<.001	1.14	1.36
Storage time	−.02	0.00	<.001	−.03	−.02
Time within patients (follow-up)	0.00	0.00	<.001	0.00	0.00
Time^2 (follow-up)	−.00	0.00	<.001	−.00	−.00
Gender	−.07	0.02	0.001	-.1.2	0.03
Age	<.00	0.00	0.001	0.001	0.004

i. SE, standard error; 95% CI, 95% confidence interval.
ii. Data are adjusted for age, gender, and time within patients^2.

As a result of the storage decay effect the TC:HDL-c ratio, that was calculated using the measured lipoprotein levels, increases as storage time increases. To better approximate the lipoprotein levels at the time the serum sample was taken, and the lipid decay correction factor was estimated. Correction for the storage decay effect will yield a lower (improved) TC:HDL ratio, which reduces the calculated CVD risk. After applying the storage correction factor, the TC:HDL-c ratio decreased. Before correction, TC:HDL ratio was classified as high in 75% of patients, compared to 54% after correction (not shown). After correction, patients moved to lower CV risk groups, as the TC:HDL-c ratio decreases after correction for the storage decay effect (Table 4). Before correction, most patients were categorized in the “low” CVD risk group (<10%) and 53 patients were reclassified from the intermediate and high risk groups to this group, totaling 552 patients (a relative 11% increase) after correction. The intermediate (10–20%) and high (>20%)

CVD risk groups decreased in size by 8% and 11% respectively. Overall, in this cohort of 1050 patients, 53 (5%) patients changed CVD risk groups according to SCORE 10-year risk predictions for CVD.

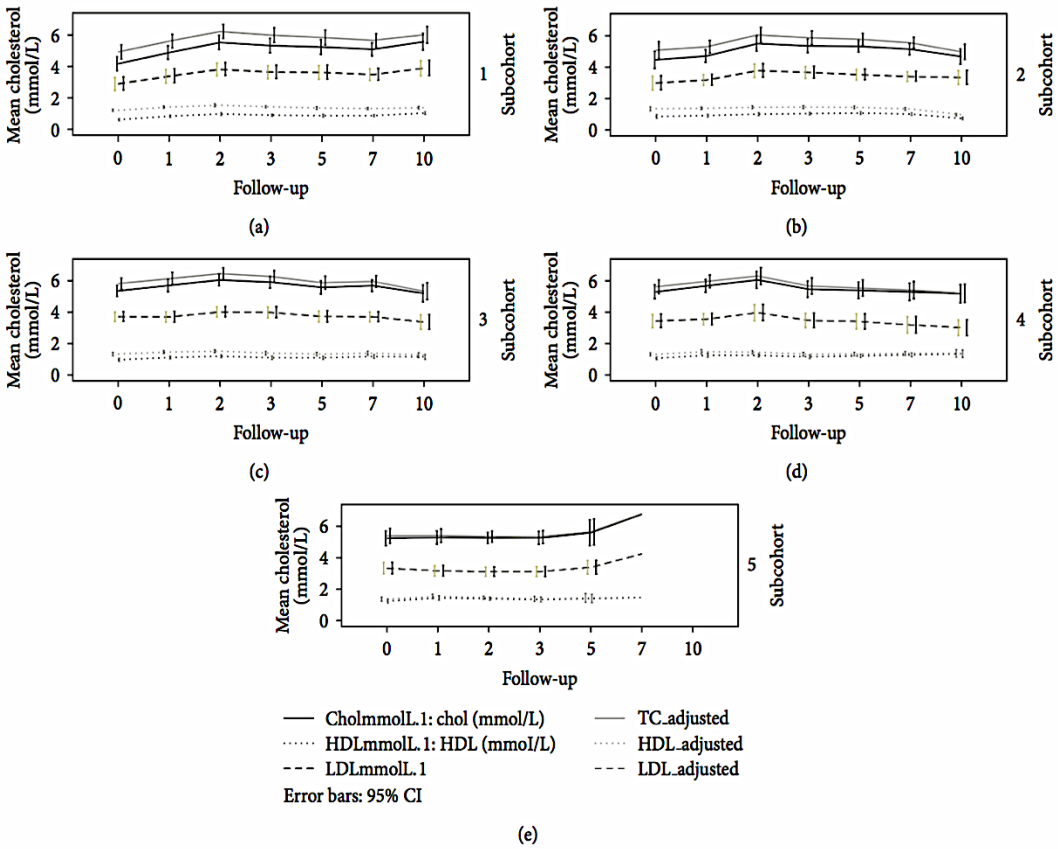

Figure 3. Lipoprotein levels measured over time in the various subcohorts before and after correction with the lipid decay factor. Mean TC and HLD-c levels are depicted on the y-axis and follow-up time on the x-axis

Table 4. Reclassification of the 10-year risk of CVD

10-year risk of CVD	Before correction for storage decay n(%)	After correction for storage decay n(%)	Reclassified (N=53)
Low (<10%)	499 (48%)	552 (53%)	+53 (11%)
Intermediate (10-20%)	178 (17%)	164 (16%)	−14 (8%)
High (>20%)	373 (35%)	334 (31%)	-39 (11%)
Total, n(%)	1050 (100%)	1050 (100%)	53 (5%)

DISCUSSION

Our study is the first to investigate the validity of cholesterol levels obtained in serum samples that have been stored for 10 years, and to evaluate if this storage decay effect is clinically important for CVD risk evaluation in RA patients. A significant but modest storage decay effect on cholesterol levels was found. The magnitude of this effect with regards to CVD risk assessment appears relatively small. Correction factors to adjust for this effect were calculated, 0.03 mmol/L for TC and 0.02 mmol/L for HDL-c, per additional year of storage. Interestingly, the absolute changes in TC and HDL-c per additional year of storage in this cohort lie close together. This means that the impact of this storage decay effect will be greater for HDL-c, as the range of HDL-c levels is much smaller compared to TC levels. The decay effect of TC levels reported in this study is less steep than the decrease in HDL-c. Therefore, the observed TC:HDL-c ratio may become increased and lead to false-higher CVD risk predictions. In particular lipid measurements in samples that have undergone long-term storage would be inaccurate, which could eventually lead to distorted risk predictions if these values were used in CVD risk assessment. However, clinical impact of the storage decay effect appears to be minimal, with only a small number of patients (5%) moving from groups indicated for primary CVD prevention (either the intermediate or high risk) to the low risk group after application of a correction factor. Although, on an individual level, changes in absolute lipid levels may be relevant, these changes do not appear to significantly affect cardiovascular risk predictions overall. Therefore, a correction factor can be used but may not be necessary if samples have been stored under stable conditions. Determining whether long-term storage affects the integrity of lipid samples requires analysis of long-term data. Ideally one would compare values determined in the past, directly after acquiring the blood sample ("original" values), with values determined from stored samples ("observed" values) within patients. The differences between "original" values and "observed" values provide an immediate indication of the storage effect. Several studies have used this approach but in serum samples that were stored for relatively short periods of 1-2 years.[19, 20, 24, 25] However, measurements of lipids directly after blood sampling are not always available in RA cohorts. This may be due to the fact that the awareness of the increased risk of CVD gained particular interest long after the start of RA cohorts. Hence a different approach is required to assess the storage decay effect on lipid levels in these cohorts.

In a study that investigated the effect of long-term storage (7 years),[26] the group means of lipids for pairs of serial specimens that were taken at 6- and 12-month intervals were compared. It was assumed that in the absence of a storage effect the variation in group means would reflect only normal biological variation, which would not lead to a systematic downward or upward effect in the group mean cholesterol levels. Subsequently, any observed changes would reflect the storage effect.[26] However, in a long running cohort that includes a lengthy follow-up of more than 10 years, patients could also be systematically different between different time-periods. For example, patients that were included more recently are probably more likely to use statins at the time the serum samples were taken than patients that were included 25 years ago. In this cohort, cholesterol levels from samples that were stored the shortest could therefore be systematically lower than samples that were stored the longest, without involvement of a storage effect. Such

a period effect could lead to biased results. Therefore, we used a method in which the data were grouped according to five different time-periods for analysis, to adjust results for a period effect. Illustratively, statin use at baseline increased from 0% in the first three subcohorts (1985–1999) to 29% in the last cohort (2005–2009). The percentage of smokers decreased from 47% to 29% during that same timeframe. Overall, these subcohorts did not seem to differ significantly, excluding period effect as a confounder in this cohort. Consequently, a simplified model was developed. The methodology used in this study can also be applied in other cohorts.

This study has limitations. The storage effect was estimated without knowing "original" values for comparison. The correction factor was directly derived from the regression coefficient of storage time. It was assumed that after correction for repeated measures and for confounders any observed change is attributable to the storage effect. Biological variation of lipid levels within a subject could potentially contribute to the found decay effect over time. However, several (n = 7) measurements per patient were included, in the total group of 152 patients. Serum samples had been stored under similar condition, albeit in different storage facilities. It was considered unlikely that any variation, either due to biological variation within patients or due to differing storage circumstances, would contribute to an overall, systemic trend. It has been previously suggested that such variability in measured cholesterol levels is unpredictable and often a wide variation in both directions (±20%) is reported.[30, 31] In the model that was formulated, the most important confounders were dealt with, but the existence of other confounding factors cannot be excluded. Storage conditions, varying temperatures during storage, and number of times the samples were thawed are all factors that could have contributed in affecting serum cholesterol levels. As multiple sets of samples from 152 patients were used, it is considered unlikely that any of these highly variable factors contributed significantly to the systemic decay effect that was found. In addition, modifying the storage temperature from −20◦C to −80◦C for all serum samples in 2008 is a limitation, although the vast majority of the samples (1985–2007) were stored under the same conditions. Storage effects are likely to be even smaller with lower temperatures.

In conclusion, the results of this study show that modest decay of lipid levels in serum samples should be expected during long-term (>10 years) storage. Using the method proposed here, a correction factor can be formulated to adjust for this storage decay effect. However, as the clinical impact on CV predictions appears minimal, extensive adjustment may not be necessary to obtain valid lipid levels. Hence, stored serum samples, even when stored for long periods of time, are a valuable source of data. These data can be of particular importance for studies in RA cohorts involving long-term outcomes such as cardiovascular disease. In addition, it may be financially attractive to minimize the analysis period by performing cholesterol measurements in stored serum samples all at once at the end of a long-term study with multiple samples taken over long periods of time. It is recommended to employ the method presented in this study in other long-term cohorts.

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CHAPTER 3

Performance of four current risk algorithms for predicting cardiovascular events in patients with early rheumatoid arthritis

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Annals of Rheumatic Diseases
2015; 74: 668-674

ABSTRACT

Objective

This study was undertaken to assess the predictive ability of four established cardiovascular risk models for the 10-year risk of fatal and non-fatal cardiovascular disease (CVD) in European patients with rheumatoid arthritis (RA).

Methods

Data from the Nijmegen early rheumatoid arthritis inception cohort was used. Discriminatory ability for CVD risk prediction was estimated by the area under the receiver operating characteristic curve. Calibration was assessed by comparing the observed versus expected number of events using Hosmer-Lemeshow tests and calibration plots. Sensitivity and specificity were calculated for the cut-off values of 10% and 20% predicted 10-year risk of CVD.

Results

Areas under the receiver operating characteristic curve were 0.78–0.80, indicating moderate to good discrimination between patients with and without CVD. The CVD risk models Systematic Coronary Risk Evaluation (SCORE), Framingham risk score (FRS) and Reynolds risk score (RRS) primarily underestimated CVD risk at low and middle observed risk levels, and mostly overestimated CVD risk at higher observed risk levels. The QRisk II primarily overestimated observed CVD risk. For the 10% and 20% cut-off values, which are used as indicators for preventive treatment, sensitivity ranged from 68–87% and 40–65%, respectively and specificity ranged from 55–76% and 77–88%, respectively. Depending on the model, up to 32% of observed first time CVD occurred in patients with RA who were classified as low risk (<10%) for CVD.

Conclusions

Established risk models generally underestimate (Systematic Coronary Risk Evaluation score, Framingham Risk Score, Reynolds risk score) or overestimate (QRisk II) CVD risk in patients with RA.

INTRODUCTION

Cardiovascular disease (CVD) risk models are informative tools guiding preventive or therapeutic strategies by providing estimations of CVD risk.[1–5] In patients with rheumatoid arthritis (RA), the risk of CVD is increased and screening of CVD risk factors and identification of high-risk patients is warranted.[6] Risk algorithms developed for the general population do not necessarily perform well in the RA population, and may underestimate the increased risk in RA. The most widely used risk algorithms are; the Framingham risk score (FRS), the Systematic Coronary Risk Evaluation score (SCORE), the Reynolds risk score (RRS) and the QRisk II risk score. The FRS has been developed and validated in American cohorts,[7] including the General Cardiovascular Risk Profile algorithm, which is the FRS adjusted to calculate the 10-year risk of fatal and non-fatal CVD.[2] The original SCORE was developed and validated in 12 European cohorts to predict fatal CVD.[1] Country-specific versions of the SCORE were developed to optimize prediction of the 10-year risk of CVD.[8, 9] SCORE and FRS are based on traditional risk factors. However, in patients with RA CVD risk is not fully explained by these factors.[10] Inflammation may account for the extra risk.[11] In the general population, inflammation has been shown to be an important independent risk factor for CVD.[12] The RRS incorporates the inflammatory marker high-sensitivity C reactive protein (hs-CRP) in addition to traditional risk factors.[4, 5] However, it is unclear if adding an inflammatory marker such as hs-CRP to a CVD risk algorithm improves the predictive performance in RA, as patients with RA may retain high levels of CRP during the course of the disease. The European League Against Rheumatism (EULAR) recommendations for CVD risk management [13] recommend the use of a multiplication factor of 1.5 to the risk estimate of the SCORE (a modified score, or M-SCORE) when a patients fulfils two out of three criteria; disease duration >10 years, rheumatoid factor or anticyclic citrullinated peptide positivity and presence of extra-articular manifestations. Recently, an updated version of the QRisk algorithm was developed by Hippisley-Cox et al.[3] This QRisk II algorithm includes RA as an independent risk factor. However, it is not known whether the M-SCORE, RRS and QRisk II risk algorithms predict future CVD in patients with RA more accurately compared with the SCORE or the FRS. The predictive performance—that is, the accuracy of predictions of future CVD event(s)—of these risk algorithms has not been evaluated and compared in European patients with RA. Therefore, the objective of this study is to assess the performance of four established CVD risk algorithms (SCORE, FRS, RRS and QRiskII) for predicting the 10-year risk of fatal and non-fatal CVD in European patients with RA.

METHODS

Study design and patients

This retrospective study is based on largely prospective collected data from the Nijmegen early RA inception cohort. Patients were included at diagnosis of RA (baseline) in the outpatient clinic of the departments of rheumatology of the Radboud University Medical Centre (since 1985) or the Maartenskliniek in Nijmegen (since 1990). At inclusion, patients had a disease duration of <1 year, were disease modifying antirheumatic drug naive and fulfilled the 1987 American College

of Rheumatology (ACR) (inclusion before 2010) or ACR/EULAR 2010 criteria (inclusion after 2010) for the classification of RA.[14] All patients provided written informed consent. Patients with a history of CVD before inclusion were excluded from our analysis. All four algorithms evaluated in this study can be used to calculate the 10-year risk of CVD. Predicted risk estimates in patients with a follow-up time <10 years were adjusted proportionally, according to the length of actual follow-up and calculated as a proportion of 10 years.[15]

Data collection

Baseline characteristics were retrieved from the cohort database including; age (years), gender (male/female), rheumatoid factor positivity, anticyclic citrullinated peptide positivity, 28-joint disease activity score and CRP (mg/L). Data on CVD risk factors at baseline were collected by medical chart and electronic patient file review, including smoking status (Y/N), blood pressure (mm Hg), use of statins (Y/N) and antihypertensive medication (Y/N), height (m) and weight (kg), diabetes mellitus (Y/N), hypertension (Y/N) and family history of CVD (Y/N). Lipid levels were measured using serum from frozen samples collected at baseline. Non-fasting total cholesterol (TC) and high-density-lipoprotein cholesterol (HDL-c) concentrations (mmol/L) were measured using laboratory facilities of Russells Hall Hospital, Dudley UK.

Primary outcome

The primary outcome is first time CVD(fatal and non-fatal), which was retrieved from physician diagnosis and extensive review of medical charts and electronic patient files. Included cardiovascular events were: acute/unstable coronary syndrome (myocardial infarction and unstable angina pectoris), stable angina pectoris, cerebral vascular accident (CVA), transient ischaemic attack, peripheral vascular disease and heart failure. Deaths due to CVD were verified from death certificates, provided by Statistics Netherlands,[16] including deaths due to CVD and CVA but excluding cerebral hemorrhage and non-coronary cardiac death (i.e., arrhythmias). As every CVD risk model has its own set of predicted outcomes, four separate outcome variables were constructed specifically adjusted to fit the models.

Risk algorithms

All risk algorithms included gender, smoking, TC:HDL-c ratio and systolic blood pressure. The 10-year general FRS for CVD [2] the SCORE [9] and RRS[4, 5] were calculated using the published risk algorithms. The SCORE that was used is the Dutch version adapted to predict fatal and non-fatal CVD.[9, 17] The QRisk II risk algorithm also includes diabetes (Y/N), atrial fibrillation (Y/N), blood pressure treatment (Y/N), RA (Y/N), body mass index (weight (kg)/height $(m)^2$), family history of CVD, chronic kidney disease and the Townsend deprivation score.[3] The latter was not available in our cohort. Therefore, CVD risk was calculated using an adjusted QRisk II algorithm excluding this variable, courtesy of ClinRisk. The M-SCORE was considered but not included because in this inception cohort it would apply only to a very small number of patients (n=23).

Statistical analysis

Baseline data were used to calculate individual estimates of the 10-year CVD risk for all four CVD risk algorithms. Missing values were imputed using multiple imputations with five repetitions. The

discriminatory ability of the four algorithms was estimated using the area under the receiver operating characteristic (ROC) curve, which is similar to the concordance-statistic (c-statistic).[18] An area under the ROC curve of 1 signifies perfect discriminatory ability and an area of 0.5 indicates the prediction model does not perform better than a random guess. Calibration was assessed by comparing the agreement between observed and predicted (calculated by means of the CVD risk algorithms) number of cardiovascular events (%) in groups of patients stratified in deciles of the predicted risk. Hosmer-Lemeshow (H-L) tests and calibration plots were used. In the calibration plots, a line was fitted between the observed and predicted probabilities of a cardiovascular event per decile of predicted risk using quadratic spline. Sensitivity and specificity were calculated for the cut-off values of 10% and 20% that mark the difference between low-risk and intermediate-to-high risk and between low-intermediate risk and high-risk patients, respectively. These cut-off points are recommended in guidelines to be used as indicators for preventive treatment; lifestyle adjustments and drug therapy interventions. In this cohort, regular CRP was measured, but values <5 mg/L were not quantified. These values were imputed as 2.5 mg/L. A sensitivity analysis was performed for CRP values below 5 mg/L using either 0 or 5 as alternatives for values <5 mg/L. All statistical analyses were performed using SPSS V.20.0.

RESULTS

Patients

In total, 1157 patients were enrolled of which 107 patients experienced a CVD event prior to the diagnosis of RA, leaving 1050 patients with 9957 patient-years for analysis. During follow-up, 149 patients developed a first cardiovascular event (1.14 events per 100 patient-years); 67 cases of acute/unstable coronary syndrome (myocardial infarction or unstable angina pectoris), 24 cases of stable angina pectoris, 26 CVAs, 10 transient ischaemic attacks, 18 cases of peripheral vascular disease and 4 cases of heart failure. Out of all these events, 15 were fatal. The primary outcome was adjusted to fit each CVD risk algorithm,[2–5 9] leaving 104, 149, 87 and 126 first cardiovascular events for analysis of SCORE, FRS, RRS and QRisk II, respectively. As the RRS is not applicable to patients with diabetes, these patients (n=44) were excluded, leaving a total of 1006 patients for analysis of the RRS. Patient characteristics for all (n=1050) patients with RA are presented in table 1. Missing values ranged from 0.1% to 10.3% at baseline for variables included in the models.

Discrimination

Discriminatory ability was comparable across the four CVD risk models. Overall, discriminative ability was good; c-statistic scores of 0.78 (95%CI 0.74 to 0.82), 0.80 (95% CI 0.77 to 0.84), 0.78 (95%CI 0.73 to 0.82) and 0.79 (95%CI 0.75 to 0.83) for the SCORE, FRS, RRS and QRisk II, respectively. The corresponding ROC curves are presented in figure 1.

Calibration

Across deciles of predicted CVD risk there were discrepancies between the observed and predicted (calculated risk scores) number of cardiovascular events for all four algorithms (figures

Table 1. Patient characteristics

	All patients (N=1050)	No CVD (N=901)	CVD (N=149)	p-value
Age (years), mean±SD	54±13.8	53±13.9	61±10.2	<.001
Female, n(%)	695 (66)	616 (68)	79 (53)	<.001
DAS28 at baseline, mean±SD	4.9±1.3	4.8±1.3	5.4±1.3	0.001
Swollen joint count, median (p25–p75)	7 (5–12)	7 (5–11)	9 (6–14)	0.002
Tender joint count, median (p25–p75)	5 (3–9)	5 (3–9)	7 (4–12)	<.001
ESR (mm/hour), median (p25–p75)	25 (16–45)	23 (16–44)	34 (20–50)	0.001
CRP (mg/L), median (p25–p75)	16 (3–42)	15 (3–41)	22 (5–47)	0.078
VAS (mm), median (p25–p75)	41 (30–57)	40 (29–55)	50 (32–64)	0.002
Rheumatoid factor positivity, n(%)	777 (74)	658 (73)	118 (79)	0.110
Anti-CCP positivity, n(%)	681 (65)	572 (64)	91 (61)	0.510
Smoking at baseline, n(%)	332 (32)	265 (29)	60 (41)	0.003
BMI (weight(kg)/Height(m)^2), mean±S	26±4.3	26±4.4	26±4.1	0.038
Systolic blood pressure (mmHg), mean±SD	146±24	145±24	155±25	<.001
Diastolic blood pressure (mmHg), mean±SD	84±12	83±12	86±10	0.004
Total cholesterol (mmol/L), mean±SD	5.1±1.2	5.2±1.2	5.3±1.4	0.127
HDL-cholesterol (mmol/L), mean±SD	1.3±0.3	1.3±0.3	1.2±0.3	0.124
TC:HDLc ratio, mean±SD	4.2±0.9	4.1±1.1	4.4±1.0	0.010
Diabetes at baseline, n(%)	44 (4)	30 (3)	14 (10)	<.001
Hypertension at baseline, n(%)	148 (14)	110 (12)	38 (26)	<.001
Treatment with antihypertensive medication at baseline, n(%)	172 (17)	144 (16)	37 (25)	0.018
Treatment with statins at baseline, n(%)	38 (4)	28 (3)	10 (7)	0.061
Family history of CVD, n(%)	326 (25)	281 (31)	53 (36)	0.386
SCORE (%), median (p25–p75)	9 (3–28)	7 (2–23)	28 (11–48)	<.001
FRS (%), median (p25–p75)	13 (5–23)	11 (5–20)	23 (15–33)	<.001
RRS* (%), median (p25–p75)	6 (2–17)	5 (1–14)	17 (7–30)	<.001
QRiskII (%), median (p25–p75)	14 (5–28)	12 (4–25)	27 (17–37)	<.001

I. For RRS, patients with diabetes are excluded; n=1006.

II. The p-value is for the difference between patients with RA with and without CVD, using two-sample t test, two-sample Wilcoxon test or $\chi 2$ test, as appropriate.

III. Anti-CCP, anti-cyclic citrullinated peptide; BMI, body mass index; CRP, C reactive protein; CVD, cardiovascular disease; DAS28, disease activity score 28-joints; ESR, erythrocyte sedimentation rate; FRS, Framingham risk score; HDL-cholesterol, high-density-lipoprotein cholesterol; RA, rheumatoid arthritis; RRS, Reynolds risk score; SCORE, Systematic Coronary Risk Evaluation; TC:HDL-c ratio, total cholesterol:high-density lipoprotein cholesterol ratio; VAS, visual analogue scale.

2 and 3). CVD risk predictions calculated using SCORE appear to deviate from the observed CVD risk in the middle and top deciles, particularly when CVD risk is underestimated (figures 2A and 3A). The H-L test yielded a p-value of <0.001, indicating poor model fit. The number of cardiovascular events predicted by the FRS were similar to the observed number of events, showing a modest difference in predicted and observed CVD risk in the lower and middle deciles. The predicted number of CVD events in the top two deciles showed a more pronounced deviation from the observed number of CVD, under- and overestimating CVD risk. The H-L test indicated

poor model fit with a p value of 0.024 (figures 2B and 3B). Overall, the CVD risk as predicted by the RRS is not in concurrence with the observed CVD risk, underestimating the number of events (figures 2C and 3C), with an overall p-value of 0.020 of the H-L test indicating poor model fit. The QRisk II mainly overestimated observed CVD risk. The H-L test result indicated moderate model fit with a p value of 0.20 (figures 2D and 3D). Overall, the number of CV events that were expected based on the risk calculated by these algorithms appeared to be an inaccurate estimate of the observed number of CVD events in patients with RA.

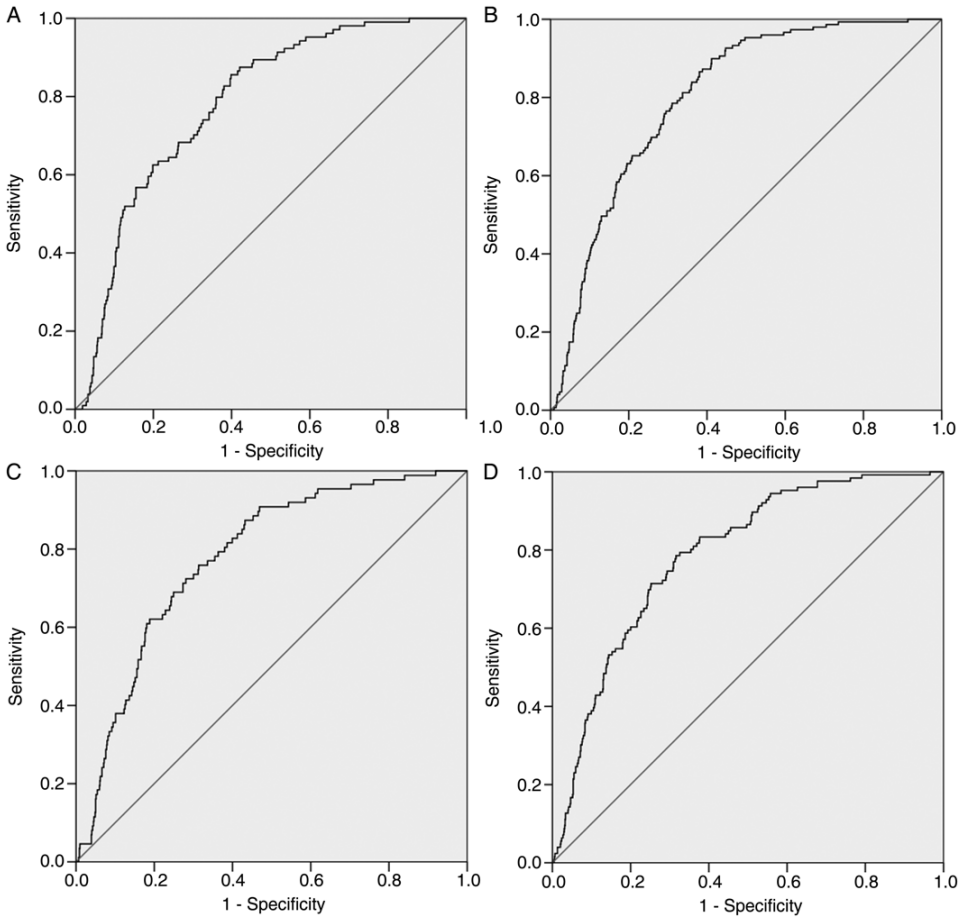

Figure 1. Receiver operating characteristic (ROC)-curves for the different risk algorithms. Area under the curve (AUC)-values (95% CI) are 0.78 (0.74 to 0.82), 0.80 (0.77 to 0.84), 0.78 (0.73 to 0.82) and 0.79 (0.75 to 0.83) for the Systematic Coronary Risk Evaluation (A), Framingham risk score (B), Reynolds risk score (C) and QRisk II (D), respectively.

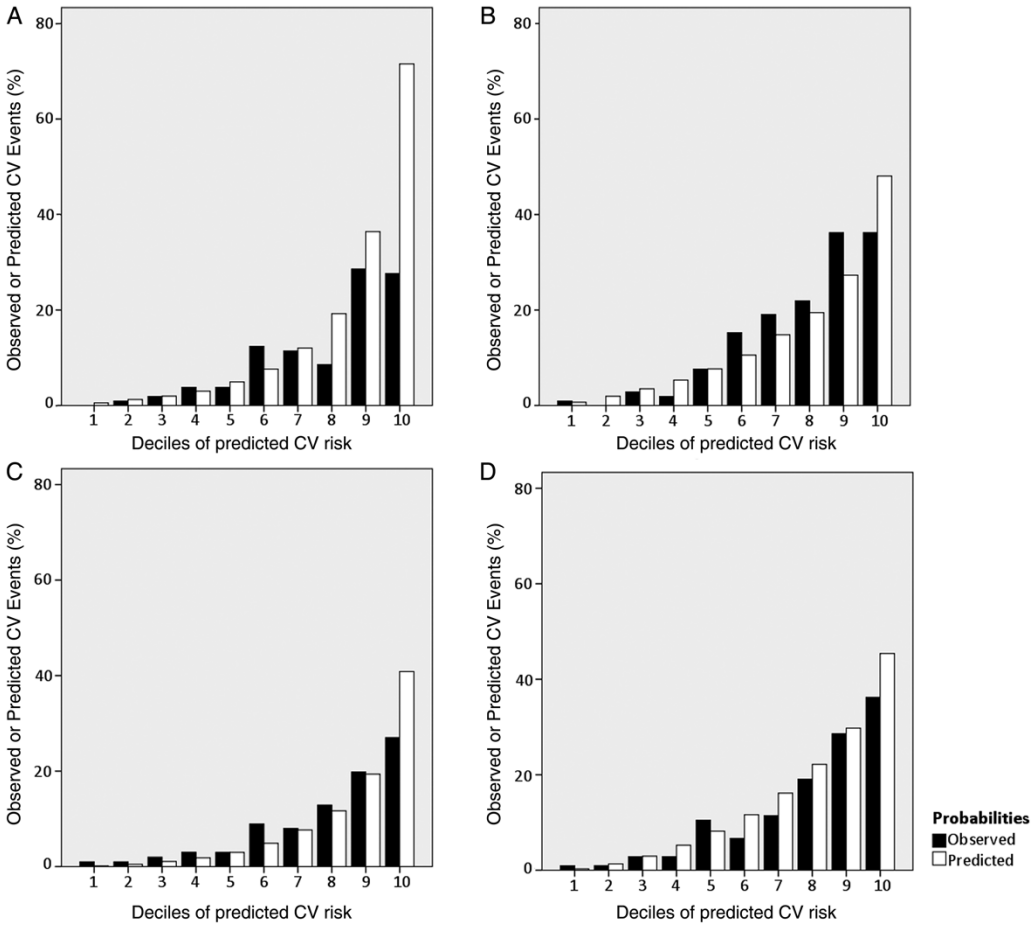

Figure 2. Observed (closed bars) versus predicted (open bars) cardiovascular (CV) events (%) in deciles of predicted risk, for the Systematic Coronary Risk Evaluation (A), Framingham risk score (B), Reynolds (C) and QRisk II (D) risk algorithm.

Sensitivity and specificity

Sensitivity and specificity of the 10% and 20% cut-off points for CVD risk are presented in table 2. Also shown in this table, are the positive and negative predictive values. The negative predictive value ranges from 92% to 97% depending on the model and the cut-off point, which indicates that out of all patients classified as being 'low risk', a relatively small number of patients did develop CVD. When considering the total number of CVD events, varying from 87–149 depending on the model, up to 24% (SCORE) and 32% (RRS) of first CVD events occurred in patients with RA who were classified as 'low risk' (<10%).

Table 2. Sensitivity and specificity of cut-off points in CV risk scores

	True case (n)	Positive test (n)	True positive (n)	False positive (n)	False negative (n)	True negative (n)	Total (%)	SENS (%)	SPEC (%)	PPV (%)	NPV (%)
SCORE											
>10%	104	410	79	331	25	615	1050	76	65	19	96
>20%	104	247	63	184	41	762	1050	61	80	26	95
FRS											
>10%	149	487	130	357	19	544	1050	87	60	27	97
>20%	149	144	88	161	61	740	1050	59	82	35	92
RRS											
>10%	87	284	59	255	28	694	1006	68	76	21	96
>20%	87	144	33	111	54	808	1006	40	88	23	94
QRisk II											
>10%	126	516	106	410	20	514	1050	84	55	21	96
>20%	126	298	82	216	44	708	1050	65	77	28	94

i. True cases represent all CV events that occurred, using the criteria for each individual risk score.
ii. The number of positive tests are the number of patients who are classified as being at intermediate-high or high risk (a risk score of >10% or >20%, respectively) for CVD, out of the total number of patients with RA.
iii. As the RRS is not applicable to patients with diabetes, these patients (n=44) are excluded, leaving a total of 1006 patients for analysis.
iv. Sensitivity is calculated as the proportion of correctly classified patients given a positive result on the outcome variable, that is, proportion of patients with a predicted risk >10% or >20% out of all the patients who experienced an event (true positive). Specificity is calculated as the proportion of correctly classified patients given a negative result on the outcome variable, that is, proportion of patients with a predicted risk <10% or <20% out of all the patients who did not have an event (true negative).
v. CV, cardiovascular; CVD, cardiovascular disease; FRS, Framingham risk score; NPV, negative predicting value; PPV, positive predicting value; RA, rheumatoid arthritis; RRS, Reynolds risk score; SEN, sensitivity; SPEC, specificity; SCORE, Systematic Coronary Risk Evaluation.

DISCUSSION

The risk estimations from the four evaluated CVD risk algorithms deviate from the observed risk; mainly overestimating (QRisk II) and underestimating (SCORE, FRS, RRS) the risk of future CV events in European patients with RA. This underestimation was most pronounced in the lower two-thirds of predicted CVD risk, in line with the underestimation of CVD by FRS and RRS in patients with RA from northern America.[15] Moreover, the low-to-intermediate range of CVD risk is most clinically relevant, as preventive interventions are recommended if CVD risk exceeds 10% or 20%. The risk models discriminated relatively well, with areas under the ROC curve of 0.78–0.80, indicating moderate to good discrimination between patients with and without CVD. Calibration of all four algorithms was poor to moderate, particularly in the clinically relevant low and intermediate ranges of risk. Out of all 'low risk' patients a relatively small number of patients developed CVD. However, these patients still accounted for up to 32% (RRS) of all CVD events and as these patients were classified as 'low risk' (underestimation) it is unlikely that they received sufficient preventative treatment. When comparing the predictive performance of the four

algorithms in patients with RA with the predictive performance reported in the general population, discriminative ability appears comparable.[3, 5, 9, 19] However, in the RA population these models appear to perform less well in terms of calibration, considering the large discrepancy between observed and predicted CVD number of events observed in this study. In the Netherlands, assessment of the SCORE risk model for the prediction of 10-year CVD mortality and morbidity showed a slight overestimation of CVD risk and relatively good discriminative ability with a c-statistic score of 0.75 in men and 0.71 in women.[9] In an American cohort, the FRS showed relatively high discriminative ability (c-statistic score of 0.76 and 0.79 for men and women, respectively) and good calibration (H-L test p value of 0.14) indicating moderate model fit.[2] The RRS showed good discrimination and calibration in the general population as well, with a c-statistic score of 0.81 and H-L test p-value of 0.61.[4] The QRisk II algorithm generated similar results in a large British cohort with a c-statistic of 0.79 and 0.82 for men and women, respectively, and good calibration.[3] These results were not achieved in the RA population of this study. This is in concurrence with a recent study by Crowson et al,[15] evaluating the RRS and FRS in 525 North American patients with RA without prior CVD reporting that both risk algorithms underestimated CVD risk. The relative importance of traditional CVD risk factors that form the foundation of these algorithms may be different in patients with RA. The selection of risk factors and the relative weight of each factor in these risk algorithms may not be a good representation of the contributing risk factors for CVD in patients with RA.

In order to better identify patients at risk, different approaches have been proposed. First, the cut-off points in CVD risk used as indications for primary prevention could be adjusted. However, this could also lead to overtreatment as the majority of patients in the lower risk group do not develop CVD. Alternatively, a correction factor could be used to adjust the CVD risk in patients with RA, as was suggested by the EULAR recommendations for CVD risk management.[13] Few patients in this cohort fulfilled two out of the three criteria (n=23) as none of the patients had a disease duration >10 years at baseline, and this model was therefore not regarded for analysis. Interestingly, recent data suggests that patients with RA may have similar chances to develop CVD early and late in their disease course.[20, 21] Another approach is the addition of RA-specific risk factors to the risk algorithm. The RRS includes CRP, a variable indicative for disease activity in RA. CRP has been associated with atherosclerosis and CVD in the general population and in the RA population.[22–27] Adding this variable to a CVD risk model could therefore improve predictive performance. However, results of this study showed that the RRS provided similar or slightly less accurate CVD risk predictions in patients with RA, compared with other models that do not include CRP. Regular CRP was used to calculate CVD risk with the RRS algorithm whereas hs-CRP is indicated,[4, 5] which could have affected the predicted probabilities. However, sensitivity analysis for CRP values <5 mg/L showed no different outcomes when setting these values at either 0 mg/L or 5 mg/L (not shown). Future research is necessary to determine whether other RA-specific baseline components that better reflect future disease activity may improve CVD risk prediction. However, simply adding disease specific parameters may not be sufficient to boost model performance without further adjusting the risk algorithm and its predictors to the RA population. Interestingly, the FRS which was developed in the US population, appears to perform

better than SCORE in this cohort of European patients, with the lowest number of patients falsely classified as being 'low-risk'. Whereas the included risk factors and predicted outcomes are quite similar between the SCORE and FRS, the relative weights attributed to the different risk factors do differ between both risk models. An explanation for these results may be that the Dutch patients with RA bear a better resemblance to the US general population (with an increased CVD burden compared with the Dutch) than to the Dutch general population, in terms of their CVD risk profile.

Several strengths and weaknesses of this study should be considered. The hs-CRP measurements were not available in this cohort which could have affected RRS performance. However, as described in the previous paragraph regarding the sensitivity analysis, this is unlikely. It was not possible to directly compare model performance between this RA cohort and a cohort of age-matched and gender-matched healthy controls. Further, these results may not be generalizable to patients with long-standing disease as an inception cohort was used. Also, the adjustment of risk scores in patients with a follow-up <10 years, may provide an inaccurate estimation if CV risk changes during the course of RA. However, evidence from our cohort suggests the risk is equal across the 10 years.[28] A strength of this study is the prospective data collection with minimal missing data. This study compares a large number of algorithms, all in the same RA cohort, which provides a comprehensive overview of CVD risk model performance in patients with RA.

In conclusion, the SCORE, RRS, FRS and QRisk II algorithms tend to mainly underestimate or overestimate CVD risk in a large portion of the RA population and provide less accurate predictions of CVD risk in the RA population, compared with results reported in the general population. Underestimating CVD risk may lead to insufficient treatment of (traditional) CVD risk factors. Perhaps, a RA-specific CVD risk model could improve CVD risk prediction in patients with RA. The performance of a RA-specific CVD risk model should be compared to the performance of the current risk algorithms.

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CHAPTER 4

High-density lipoprotein cholesterol subfractions HDL2 and HDL3 are reduced in women with rheumatoid arthritis and may augment the cardiovascular risk of women with RA: a cross-sectional study

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Arthritis Research & Therapy
2012: 14; R116

ABSTRACT

Introduction

Higher levels of high density lipoprotein cholesterol (HDL-c) subfractions HDL3-c and particularly HDL2-c protect against cardiovascular disease (CVD), but inflammation reduces the HDL-c level and may impair its anti-atherogenic effect. Changed HDL-c composition through the impact of inflammation on HDL-c subfractions may contribute to the excess risk of CVD in rheumatoid arthritis (RA). In this study, we investigated whether HDL2-c and HDL3-c concentrations differ between RA patients and healthy controls, and whether these levels are related to the level of RA disease activity.

Methods

Non-fasting blood samples were collected from 45 RA patients and 45 healthy controls. None of the participants had a history of CVD, diabetes, or used lipid-lowering drugs. HDL2-c and HDL3-c concentrations were obtained by ultracentrifugation. Regression modeling was used to compare HDL-c subfraction levels between RA patients and healthy controls, and to analyze the effect of disease activity on HDL2-c and HDL3-c.

Results

HDL2-c and HDL3-c were significantly lower in RA patients compared to healthy controls (P = 0.01, P = 0.005, respectively). The HDL2-c:HDL3-c ratio was significantly lower in patients compared to controls (P = 0.04). Reduced HDL2-c and HDL3-c levels were primarily present in female RA patients and not in male RA patients. A modest effect of the 28-joint disease activity score (DAS28) on HDL2-c concentrations was found, after correction for disease duration, glucocorticosteroid use and body mass index (BMI), with a 0.06 mmol/L decrease with every point increase in DAS28 (P = 0.05). DAS28 did not significantly affect HDL3-c concentrations (P = 0.186).

Conclusions

Both HDL-c subfractions but particularly HDL2-c concentrations were decreased in RA, primarily in women. This seems to be associated with disease activity and is of clinical relevance. The reduction of the HDL-c subfraction concentrations, particularly the supposedly beneficial HDL2-c, may negatively impact the cardiovascular risk profile of women with RA.

INTRODUCTION

Cardiovascular morbidity and mortality are increased in the rheumatoid arthritis (RA) population.[1-3] The risk of cardiovascular disease (CVD) in RA is approximately two- to three-fold greater than in the general population, comparable to the risk of patients with type 2 diabetes mellitus.[4] As traditional risk factors do not fully account for the increased CVD risk in RA, it is suggested that inflammation plays an important role in mediating cardiovascular risk in these patients.[5,6] In RA, it has been shown that inflammation affects the lipid profile and accelerates atherosclerosis.[7,8] However, it appears that there is no difference in risk of CVD between patients with low or high disease activity.[9] Apparently, low levels of inflammation are sufficient to increase CVD risk in RA. In the general population, increased levels of total cholesterol (TC), low-density-lipoprotein cholesterol (LDL-c) and triglycerides, and decreased levels of high- density lipoprotein cholesterol (HDL-c) or a pro-atherogenic lipid profile, are important risk factors for CVD.[10] In the general population, HDL-c is regarded as the main anti-atherogenic lipoprotein and increased levels of HDL-c have been associated with a protective effect against cardiovascular mortality and morbidity.[11,12] The beneficial effect of HDL-c appears to be the strongest for women.[12] This advantageous effect of HDL-c is supposed to be accomplished primarily through the reverse cholesterol transport (RCT) and the neutralization of oxidized lipids.[13] In RA patients, however, the effect of changes in lipid concentrations on CVD risk is less straight forward.[8] Lipoprotein and apolipoprotein levels are known to fluctuate during the course of RA, possibly under the influence of inflammation and anti-inflammatory treatment, including oral steroids and biologic therapies.[14-17] During active disease, increased levels of TC, triglycerides (TG) and apolipoprotein B (ApoB), and reduced concentrations of HDL-c have been reported.[14] Other aspects of the lipid profile may be of importance. The inflammatory response in RA patients may compromise the beneficial anti-atherogenic effect of HDL-c on CVD risk. In addition to lower levels of HDL-c,[14,18,19] inflammation may reduce the anti-oxidative capacity, impair RCT capacity of HDL-c in RA patients, and even lead to HDL-c becoming pro-atherogenic.[20-23] The functionality of HDL-c is partially dependent on HDL-c composition. Based on its density HDL-c can be divided into two main subfractions: HDL2-cholesterol (HDL2-c) and the smaller HDL3-cholesterol (HDL3-c). HDL2-c has been suggested to be the more variable component of total HDL, while primarily higher levels of the HDL2-c subfraction contribute to the anti-atherogenic effect of HDL-c.[24-26] For that reason, decreased levels of HDL-c subfractions and particular HDL2-c could contribute to the risk of CVD in RA. However, it is currently unclear whether HDL2-c and HDL3-c concentrations are actually decreased in RA, and whether their levels are associated with the level of disease activity. Therefore, the objective of this study was to investigate whether HDL2-c and HDL3-c concentrations differ between RA patients and healthy controls, and to investigate whether HDL2-c and HDL3-c concentrations are associated with the level of RA disease activity.

METHODS

Patients and controls

This is a cross-sectional study comparing a group of 45 consecutive patients with RA, with a control group of 45 consecutive healthy individuals. RA patients were included in the study if they fulfilled the 1987 American College of Rheumatology classification criteria for RA, did not use lipid-lowering drugs, had no cardiovascular comorbidity (hypertension was allowed) or diabetes. Disease activity scores (DAS28) were registered. The controls were consecutively recruited from healthy blood-donors who came to the regional blood bank for a donation and were matched for gender. The donors did not suffer from any inflammatory or auto-immune disorders and did not use lipid-lowering drugs, and were free of cardiovascular comorbidity and diabetes. All patients and controls who were entered in the study provided their informed consent. The Medical Ethical Committee Arnhem-Nijmegen approved the study. An a priori sample size calculation was deemed inappropriate, as evidence regarding HDL2-c and HDL3-c concentrations is limited and no studies have been performed investigating these concentrations in RA patients.

Lipoprotein measurements

In both patients and controls, non-fasting blood samples (30 ml) were collected, using vacutainer tubes (Beckton Dickinson, Rutherford, New Jersey, USA) containing K3-ethylenediaminetetraacetic acid (EDTA) (1 mg/ml), and a sample was taken in a tube without anticoagulant to obtain serum. Tubes were centrifuged at 3,600 rpm for 10 minutes at 23°C, and frozen at -80°C until assay. Levels of plasma TC, TG and HDL-c were determined enzymatically on an Eroset Hitachi 747 analyzer which was validated for these particular measurements. During each run, samples from both patients and controls were analyzed to prevent any within and between run variability to affect group differences in HDL-c subfraction levels. Low-density lipoprotein (LDL) cholesterol levels were calculated according to the Friedewald formula, which provides reliable values up to a TG concentration of 4.0 mmol/L. ApoB and ApoA-I levels (mg/L) were determined by immunonephelometry. HDL-c subfractions were isolated by means of ultracentrifugation.[27] In short, 2 ml plasma was introduced in MSE (Measuring and Scientific Equipment) tubes of the ultracentrifuge (Beckman L7-55). KBr and CBBR (Coomassie Brilliant Blue R) (1.5% solution) was added and the tubes were centrifuged at 44,000 rpm for 22 hours at 15°C. HDL2-c (density 1.08 g/ml) and HDL3-c (density 1.149 g/ml) were separated and their concentrations were measured as previously described for total HDL-c, correcting for density and volume (2 ml).

Statistical analyses

The primary outcome was the difference in the concentration of HDL2-c (mmol/L) between RA patients and the control group. Secondary outcomes were between-group differences in HDL3-c (mmol/L), HDL2-c:HDL3-c ratio, TC, TG, HDL-c, LDL-c (mmol/L), ApoA-I and ApoB (mg/L) levels. The differences in HDL2-c and HDL3-c concentrations and lipoprotein levels (TC, TG, LDL-c, HDL-c, ApoA-I and ApoB) between RA patients and healthy controls were analyzed using independent-sample t-tests, with $\alpha = 0.05$. To analyze the influence of age and gender on HDL2-c and HDL3-c concentrations, analysis of covariance was used with HDL2-c or HDL3-c concentrations (mmol/L)

as the dependent variable, RA (patient or control) as independent variable, and gender and age as covariates and by including an interaction term for gender and presence of RA. To analyze the effect of disease activity on the HDL2-c and HDL3-c concentrations, regression analysis was used with HDL2-c or HDL3-c concentrations as the dependent variable, disease activity as the independent variable, and age, gender, smoking, BMI, rheumatoid factor positivity and disease duration as potential confounders. Additional analyses were performed to investigate the effect of the inflammatory markers erythrocyte sedimentation rate (ESR; mm/hour) and C-reactive protein (CRP; mg/L) included in the DAS28. Measured values of CRP levels <5 mg/L were set at 0 mg/L. Potential confounders were added stepwise to the model, with a change in the regression coefficient of disease activity of at least 10% as the selection criterion. Mean HDL2-c and HDL3-c levels were determined for patients treated with glucocorticosteroids, methotrexate and biologicals separately and compared by means of nonparametric statistics.

RESULTS

Patients and controls

A total of 13 men and 32 women with RA were included with a mean ± standard deviation (SD) age of 58±10 years and 60±11 years, respectively. The same number of healthy controls (n = 45) were included, 13 men and 32 women with a mean±SD age of 54±6 years and 55±8 years, respectively. The participants in the control group were on average 5 years younger than the patients in the RA group (Table 1). Patient characteristics are presented in Table 1.

Table 1. Patient characteristics

	RA patients (n = 45)	Healthy controls (n = 45)
Age, mean±SD (years)	60±10.1	55±7.8
Female, n(%)	32 (71)	32 (71)
Rheumatoid factor positive, n(%)	40 (89)	N/A
DAS28, mean±SD	3.1±1.7	N/A
ESR, median (P25 to P75)	14 (5 to 28.5)	N/A
CRP, median (P25 to P75)	0 (0 to 12)	N/A
Smoking, n(%)	16 (32)	–
BMI, mean±SD	25±(3.3)	–
Anti-rheumatic medication, n(%)	15 (33)	N/A
Methotrexate	22 (49)	N/A
Other DMARD	11 (24)	N.A
Biological DMARD	15 (33)	N/A
Oral glucocorticoids, n(%)	8 (18)	N/A

i. BMI, body mass index; CRP, C-reactive protein; DAS28, disease activity score (28 joints); DMARD, disease modifying anti-rheumatic drugs; ESR, erythrocyte sedimentation rate; n, number.

Lipid and lipoprotein patterns in RA and controls

HDL-c subfractions HDL2-c and HDL3-c, and other lipid and lipoprotein levels, were compared between the RA and healthy control group. Results are presented in Table 2. The recovery of HDL-

c after separation into subfractions averaged 95%. The results show that TC and LDL-c levels did not differ significantly, while TG and Apo B levels were significantly higher in RA, and HDL-c and Apo A-1 levels were lower (Table 2). Notably, both HDL-c sub fractions HDL2-c (P = 0.01) and HDL3-c (P = 0.005) were significantly reduced in RA patients (Figure 1). Results regarding HDL2-c concentrations demonstrated a larger difference between RA patients and controls, compared to HDL3-c, and consequently the HDL2-c:HDL3-c ratio also was significantly lower in RA patients (Table 2).

Table 2. Results from the independent-sample t-test of lipid and lipoprotein levels in RA patients and healthy controls.

	RA Patients (n=45)	Healthy controls (n=45)	P-value
Lipids (mmol/L), mean±SD			
TC, mean±SD	5.7±1.1	5.7±0.8	0.72
TG, mean±SD	2.1±1.2	1.6±0.8	0.04
HDL-c, mean±SD	1.3±0.3	1.6±0.3	<.001
HDL2-c	0.5±0.3	0.7±0.4	0.01
HDL3-c	0.8±0.2	0.9±0.2	0.005
HDL2-c:HDL3-c	0.5±0.3	0.7±0.4	0.04
LDL-c, mean±SD	3.5±0.9	3.3±0.7	0.41
Apolipoproteins (mg/L)			
Apo A-1, mean±SD	1517.2±242.2	1710±217.2	<.001
Apo B, mean±SD	987.5±220.3	862.2±188.8	0.005

i. Apo, apolipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; n, number; RA, rheumatoid arthritis; SD, standard deviation; TC, total cholesterol; TG, triglycerides.

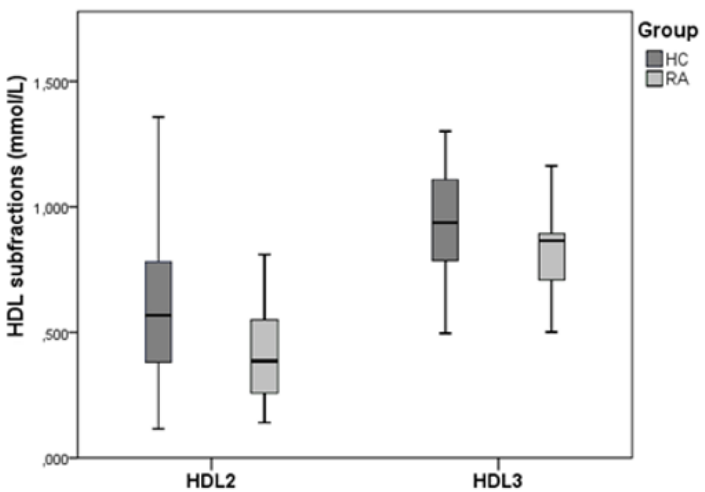

Figure 1. HDL2-c and HDL3-c subfractions in RA patients (RA) and healthy controls(HC)

Influence of age and gender on HDL2-c and HDL3-c concentrations.

There appeared to be no association between age and each of the HDL-c subfractions HDL2-c and HDL3-c (not shown). In healthy controls, both HDL2-c (P < 0.001) and HDL3-c (P < 0.001) levels were significantly higher in women. In RA patients, these levels were not different between men and women (P = 0.13 and P = 0.20, respectively). Therefore, the difference between RA and healthy controls was analyzed for men and women separately. In men, the differences between RA and healthy controls in HDL2-c (0.011 mmol/L, 95% confidence interval (CI) -0.17 to 0.15) and HDL3-c (0.007 mmol/L, 95%CI -0.16 to 0.18) were not significant (P = 0.89 and P = 0.93, respectively) (figure 2). For women, these differences between RA patients and controls in HDL2-c (0.27 mmol/L, 95%CI 0.10 to 0.45) and HDL3-c (0.16 mmol/L, 95%CI 0.08 to 0.23) were significant (P = 0.003 and P < 0.001, respectively). After repeating the analyses using linear regression with an interaction term for group and gender (see methods), it appeared that there were interactions regarding gender and presence of RA for HDL2-c (P = 0.055), as well as for HDL3-c (P = 0.063). In women, the HDL2-c:HDL3-c ratio was significantly different between RA and controls, with a mean difference of 0.2 (95% CI 0.04 to 0.40, P= 0.02) compared to a mean difference of 0.01 (95% CI -0.2 to 0.1, P = 0.9) in men. These findings are mirrored in TG, Apo A-I and Apo B concentrations. TG and Apo B levels were significantly increased only in female RA patients compared to female controls (P = 0.002 and P = 0.005, respectively). Apo A-1 levels were found to be significantly lower in female RA patients with a mean difference of 229.8 mg/L (P < 0.001) and similar in men with a mean difference of 88.6 mg/L (P = 0.379).

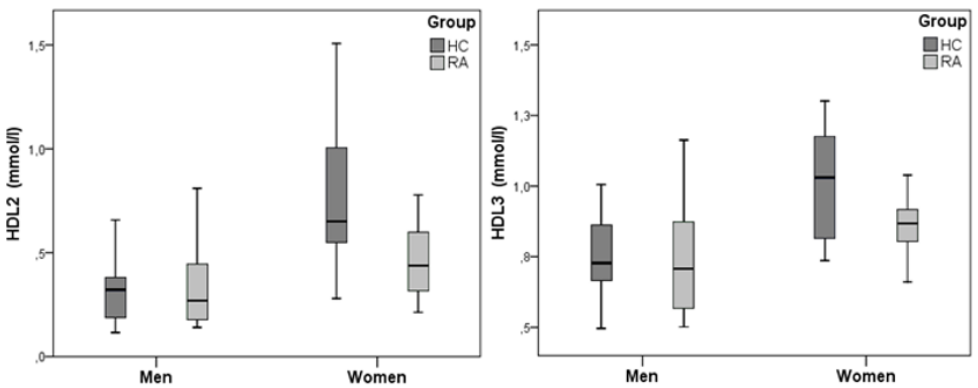

Figure 2. HDL2-c and HDL3-c subfractions in male and female RA patients (RA) and healthy controls (HC).

HDL2-c, HDL3-c and disease activity in RA

Figure 3 shows that, in RA patients the HDL2-c and HDL3-c concentrations were similar across all categories of the DAS28 (low <3.2; n = 31, medium 3.2 to 5.1; n = 6, and high >5.1; n = 8). The effect of disease activity, as a continuous measure, on HDL2-c concentrations was estimated using regression analysis, with gender, age, disease duration and use of glucocorticosteroids as covariates, while smoking, BMI and rheumatoid factor were not acting as confounders. A statistically small effect of DAS28 on HDL2-c was found (Figure 3). HDL2-c concentration

decreased by 0.06 mmol/L with every point increase in DAS28 (P = 0.05), while correcting for gender, age, disease duration and use of glucocorticosteroids. Disease activity did not significantly relate to HDL3-c, which decreased by 0.02 mmol/L with every point increase in DAS28 (P = 0.19), while correcting for gender, age, disease duration and use of glucocorticosteroids. The inflammatory marker ESR was significantly associated with HDL2-c (P = 0.046) and HDL3-c (P = 0.006), while correcting for gender, age, disease duration and use of glucocorticosteroids. (CRP did not have a significant effect on either HDL2-c or HDL3-c levels (P = 0.51 and P = 0.21, respectively). The effect on HDL2-c and HDL3-c for every point increase in ESR (mm/hour) and CRP (mg/L) was small; a decrease of 0.006 mmol/L and 0.002 mmol/L, respectively, in HDL2-c, and a decrease of 0.004 mmol/L and 0.002 mmol/L, respectively, in HDL3-c.

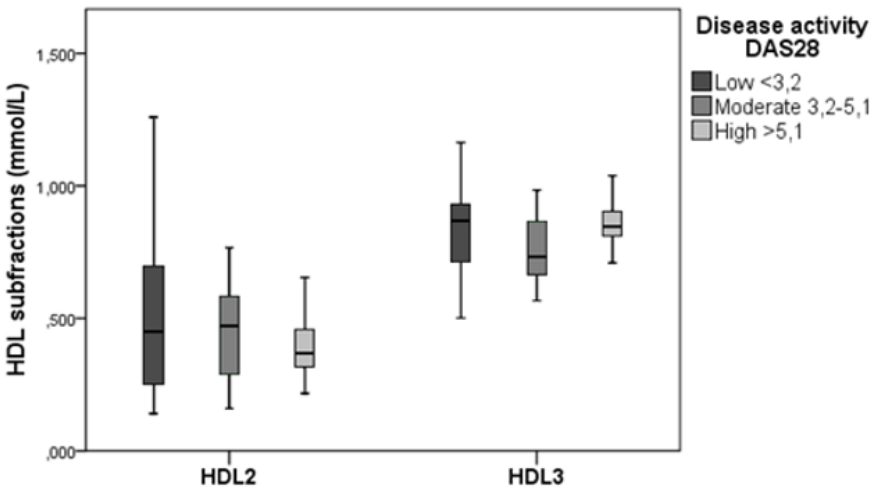

Figure 3. HDL2-c and HDL3-c concentrations in a group of RA patients with low, moderate and high disease activity levels.

HDL2-c, HDL3-c in relation to treatment and disease duration in RA

Use of (oral) glucocorticosteroids was included as a confounder in the analysis of the effect of disease activity on HDL2-c and HDL3-c concentrations. The mean±SD HDL2-c and HDL3-c levels in users and non-users of methotrexate, biologicals and glucocorticosteroids are presented in Table 3. The subanalysis of HDL2-c and HDL3-c levels in non-users and users of glucocorticosteroids (at the time of the study) showed moderately lower mean concentrations of HDL2-c and moderately higher HDL3-c levels in users (Table 3), without reaching statistical significance. Glucocorticosteroid use and mean prescribed dosage was similar in men and women (not shown). In patients with a longer disease duration (≥10 years) versus patients with a shorter disease duration (<10 years), higher levels of both HDL2-c (mean±SD 0.6±0.4 versus 0.3±0.1) and HDL3-c (mean±SD 0.9±0.1 versus 0.7±0.2) concentrations were found. Interestingly, in this study, women had a longer mean±SD disease duration (18±11 years versus 8±7 years in men) and a higher mean±SD DAS28 score (3.4±1.8 versus 2.4±1.2).

Table 2. Results from the independent-sample t-test of lipid and lipoprotein levels in RA patients and healthy controls.

	Methotrexate			Biologicals			Glucocorticosteroids		
	Yes	No	p-value	Yes	No	p-value	Yes	No	p-value
HDL2-c, (mmol/L) mean±SD	0.47 ± 0.4	0.46 ± 0.3	0.70	0.42 ± 0.2	0.49 ± 0.4	0.83	0.43 ± 0.2	0.48 ± 0.4	0.87
HDL3-c, (mmol/L) mean±SD	0.82 ± 0.2	0.83 ± 0.2	0.94	0.82 ± 0.2	0.84 ± 0.2	0.36	0.86 ± 0.1	0.81 ± 0.2	0.34

i. high density lipoprotein; RA, rheumatoid arthritis; SD, standard deviation.

DISCUSSION

This study is the first to investigate the distribution of HDL-c subfractions in RA patients. According to our results, both HDL-c subfractions but particularly HDL2- c concentrations were decreased in RA, leading to a decreased HDL2-c:HDL3-c ratio in these patients. Intriguingly, the differences in HDL-c subfractions between RA and controls were most evident in women, whereas similar levels of HDL2-c and HDL3-c were observed in men. Disease activity was not strongly related to the level of HDL2-c or HDL3-c. Finally, our results suggest that the low HDL2-c concentration might contribute to the previously reported increased cardiovascular risk in RA women. Results from previous studies investigating HDL-c and its anti-atherogenic properties in inflammatory conditions such as RA, indicate that HDL-c function deteriorates and may even become pro-atherogenic in these patients.[20-23] An inverse relation between HDL3-c and risk of CVD has been previously reported.[28,29] When comparing HDL-c subfraction levels in cases with CVD and controls the largest differences were found in HDL2-c concentrations, and often stronger inverse associations between HDL2-c and CVD were reported.[24-26,30] This is in accordance with our results; the largest difference between RA patients and controls was found in HDL2-c concentrations, and, consequently, the HDL2-c:HDL3-c ratio was lower in this group. This fact might translate into an impaired RCT, one of the crucial anti-atherogenic mechanisms involving HDL-c. The RCT relies on the quantity of both HDL2-c and HDL3-c. Several enzymes, including cholesterylester transfer protein (CETP) may affect HDL2-c and HDL3-c concentrations, by lowering them.[13] Interestingly, higher CETP concentrations have been previously indicated in RA patients providing a possible explanation for the decreased HDL2-c levels observed in our study.[31] Lower HDL2-c levels may impair RCT in these patients, contributing to accelerated atherosclerosis. Nevertheless, future research is necessary to clarify the exact mechanisms responsible for the differences in HDL-c subfractions between RA patients and controls. In our study, HDL2-c and HDL3-c concentrations were lower in men compared to women in both groups investigated. This is in accordance with previous findings in the general population. Interestingly, the differences in subfraction levels that were found between the RA patients and healthy individuals were only apparent in women, whereas no such differences between male RA patients

and healthy controls were seen. It has been previously suggested that the excess risk of CVD in RA is primarily attributable to female RA patients.[32,33] Compared to the general population, the mortality rate in RA is increased in both men and women, but mortality from all cardiac causes is larger in women than in men.[34] Hence, it is tempting to speculate that the excess CVD risk in RA women might be partly due to the relative reduction in the beneficial HDL subfraction HDL2-c. This is supported by previous findings that show a decrease in HDL-c and HDL2-c concentrations in postmenopausal women compared to premenopausal women,[35] a transition that is reported to induce an increase in the risk of CVD, although modest, supposedly due to a decrease in endogenous estrogen.[36-38] Nevertheless, it is unlikely that serum estrogen decisively contributed to the differences in HDL2-c observed in the present study, as it has been demonstrated to be unaltered in RA women.[39] Alternatively, some differences in RA-related parameters between men and women may have contributed to this result. In line with that, we observed that RA women in this study had longer disease duration and higher mean disease activity. However, there is inconsistent evidence of a certain influence of cumulative disease activity on the lipid profile in RA patients. We have previously shown that disease duration is not strongly associated with cardiovascular risk.[9,40] Others, in turn, have indicated that disease duration is associated with accelerated atherogenesis and evidence of increased carotid intima-media wall thickness [41] or presence of carotid plaques.[42] Further research is needed in order to shed more light into this interesting issue. Our results show that in the RA group, there is a modest association between DAS28 and HDL2-c concentration and no apparent relationship between DAS28 and HDL3-c concentrations. For each 1.0 increment of the DAS28, HDL2-c decreased by 0.06 mmol/L. If disease activity in a patient increases from a very low to a high DAS28 score by 3.1 points, from 2.0 to 5.1, theoretically the HDL2-c concentration would decrease 0.19 mmol/ L. Although on a statistical level these differences did not prove to be significant, it is likely that such an effect, if sustained for a longer period of time, would become clinically relevant.[43] Hence, successful suppression of disease activity by means of treatment may also have a clinically relevant effect on risk of CVD by augmenting HDL2-c and HDL3-clevels. Due to the limited number of RA patients who were investigated, we were unable to find consistent differences between the various treatment strategies regarding HDL subfraction levels. This is an interesting issue to pursue in future research.

There are several limitations of this study that require consideration. Data regarding variables that could be possible confounders in the analysis of lipid concentrations, such as smoking and BMI were not available for the healthy controls and were, therefore, not included in the analysis. Although it is unlikely that differences in these variables between RA patients and controls are of sufficient size to yield biased results, patients and controls were matched on age and gender and randomly selected to prevent selection bias. Also, this is a cross-sectional study and, therefore, no long-term effects are reported. Hence, prolonged exposure to high disease activity or remission over time and the possible effect on HDL-c composition as well as possible changes in HDL-c composition before and during the course of RA require further research. Further, lipid measurements were performed using non-fasting blood samples, possibly affecting cholesterol levels. However, HDL-c concentrations are less likely to be affected.[44,45]

In conclusion, levels of HDL-c and both HDL2-c and HDL3-c but particularly HDL2-c, were decreased in female RA patients but not in male patients, compared to healthy controls. This also affected the HDL2:HDL3 ratio, which decreased in RA. Disease activity level seems to be related to the level of HDL2-c and HDL3-c albeit in a modest association. This abnormal HDL-c subfraction pattern may negatively impact the risk of CVD in RA patients, particularly women. Larger prospective studies would be further necessary in order to test this hypothesis.

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CHAPTER 5

Atherogenic index and high-density lipoprotein cholesterol as cardiovascular risk determinants in rheumatoid arthritis: the impact of therapy with biologicals

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Mediators of inflammation
2012; doi 10.1155/2012/785946

ABSTRACT

Cardiovascular diseases (CVD)are a serious concern in rheumatoid arthritis (RA), accounting for approximately one-third to one-half of all RA-related deaths. Besides the attempts to identify new risk factors, the proper management of traditional CVD risk factors such as dyslipidemia should become a priority in the periodic evaluation of every RA patient. Atherogenic index has been suggested to be less susceptible to disease activity variation during large periods of time, making him more attractive to be used in CVD risk prediction in this group of patients as compared to individual lipids concentrations. Nevertheless, inflammation may negatively impact the anti-atherogenic properties of high-density lipoprotein cholesterol (HDL-c), suggesting that HDL-c function assessment is of particular importance when predicting CVD risk in these patients. Tight control of inflammation becomes therefore crucial for a successful CVD risk management. The present paper debates these hypotheses focusing on the effects of therapy with biologicals on the above-mentioned parameters.

INTRODUCTION

Cardiovascular disease (CVD) is a serious concern in patients with chronic inflammatory diseases. For patients with rheumatoid arthritis (RA), it represents the leading cause of death, accounting for approximately one third to one half of all RA-related deaths.[1, 2] In order to decrease this incidence, risk factors need to be identified. Intriguingly, previous studies have suggested that the augmented CVD burden found in RA patients is not fully explained by traditional CVD risk factors, such as dyslipidemia, hypertension, smoking, and physical inactivity.[3] Consequently, factors leading or deriving from chronic inflammation have been suggested to be responsible for the augmented CVD risk.[4–6] However, no such factor is proved to definitively confirm this hypothesis. Recently, several studies have suggested that CVD risk assessment in RA patients can be improved solely by focusing on the traditional risk factors. Impaired during the periods of active disease, physical activity could be significantly improved by better disease control as suggested in the recent international guidelines.[7] Using different methods to assess the risk of developing CVD, Toms et al. have recently reported that between 2% and 25% of RA patients who should receive a lipid lowering drug (statin) according to their calculated risk do not actually use this medication.[8] The percentages may even increase from 7% to 30% if the 1.5 multiplier factor is applied as recently recommended.[9] Despite its limitations, the study emphasizes the possibility of suboptimal therapy of traditional risk factors in RA patients, providing a means of reducing CVD risk in RA. Furthermore, inflammation may alter traditional CVD risk factors including the lipid pattern, by augmenting concentration and composition level.[10, 11] This observation has recently led to the concept of “smaller slice of a bigger pie,” which emphasizes that due to the presence of chronic inflammation, the relative contribution of these factors to overall CVD risk in RA is different than in the general population. All these data suggest that despite the progresses made in the past years, traditional CVD risk factors such as dyslipidemia are not yet entirely understood and appropriately managed in patients with RA. Traditionally, the atherogenic lipid profile is made up of increased total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), triglycerides (TG), and decreased high-density lipoprotein cholesterol (HDL-c). In chronic inflammatory diseases, such as RA, however, different concentrations of lipids can be found throughout different stages of the disease: increased TC and LDL-c in the years prior to disease onset, reduced levels of TC and HDL-c during early active disease, and different patterns in established RA.[12, 13] Hence, due to the variable degree of chronic inflammation, the individual lipid concentrations may frequently fluctuate during the course of disease making the impact of such changes on CVD risk less clear. Nevertheless, the different cholesterol fractions seem to fluctuate together in the same direction. In line with this, recent studies have suggested that the atherogenic index (AI—the ratio TC:HDL-c) is less susceptible to disease activity fluctuations in RA. Therefore, one can hypothesize that AI may be more appropriate to be used in CVD risk assessment in RA patients than individual cholesterol fractions measurements. Finally, inflammation may not only modulate lipid levels but also change the composition of lipoproteins. In line with this, our group and others have shown that HDL-c becomes less anti-atherogenic in RA patients, and this is associated with inflammatory status.[10, 11] Therefore, we suggest that in chronic inflammatory conditions, HDL-c anti-atherogenic properties (i.e., antioxidant, cholesterol reverse transport) may prove to be a valuable alternative marker to predict the

development of atherosclerosis and CVD in RA patients. Recent recommendations for the treatment of RA propose a tight control of disease activity to achieve rapid remission in the early disease stage. Controlling the inflammatory process is likely to favorably impact CVD risk. In line with this, new therapeutic strategies have been recently elaborated, encouraging the use of aggressive anti-rheumatics, including biologicals, earlier in the course of disease.[7] The consequence will be that an increasing number of RA patients will be treated in the future with these drugs. Appropriate knowledge about their effects on cardiovascular risk factors, including lipid pattern, would therefore be of great importance. Several previous publications have addressed the effects of biologicals on the lipid profile, concentrating on individual lipid levels/changes. However, important questions regarding the overall atherogenic capacity of the lipid profile and the subsequent impact on the cardiovascular risk remain largely unanswered. The present paper focuses on the relation between the therapy with biologicals and atherogenic index as a more suitable parameter in RA to address CVD risk in this population. In addition, data on HDL-c function in the same context will be discussed.

Methods

Literature Search and Study Selection

We conducted a literature search in Medline via PubMed for articles published up to May 2012. The MeSH terms used were anti-TNF, infliximab, adalimumab, etanercept, tocilizumab, rituximab, and rheumatoid arthritis (MeSH). These were combined with cholesterol (MeSH), lipids, HDL, and atherogenic index. Articles were selected if they met all of the following criteria: (a) clinical trial or observational study that included ≥10 patients with rheumatoid arthritis (except for rituximab studies), (b) treatment with infliximab, adalimumab, etanercept, tocilizumab, or rituximab, and (c) values of total cholesterol (TC), HDL-c, and atherogenic ratio's taken before and after treatment. The search was further restricted to English language full-text articles. Studies were manually selected by two authors (CP, EA) by screening the title, keywords, and abstract, using the eligibility criteria. If possibly eligible, full-text articles were retrieved and judged using the eligibility criteria. The inclusion of articles was determined by consensus.

Data Presentation.

Due to the heterogeneity of study populations, type of treatment, dosages, follow-up time, outcome measures, and statistical analysis, a meta-analysis was not performed. Hence, a narrative summary of the results is provided. The primary summary measure used to compare results was the difference in AI for short-term studies (<6 months) and long-term studies (>6 months). Results regarding anti-TNFα, anti-IL-6R, and anti-CD20 therapy are discussed. No additional quality assessments were performed. Sample size, differences in type of treatment and dosages, and study duration were taken into consideration when comparing results.

RESULTS & DISCUSSION

In total, there were 105 records identified. Of them, 4 were excluded because they were not written in English, 5 were case reports, 56 were off topic, 3 were themselves reviews, and 4 studies investigated less than ten RA patients (see inclusion criteria). At the end of the selection procedure, 33 full-text articles met the eligibility criteria and were considered for this paper (figure 1). Of the 33 studies, the vast majority concerned anti-TNF users, usually infliximab, adalimumab, and etanercept,[11, 14–32] 8 studies concerned tocilizumab (including three randomized clinical trials),[21, 33–39] and 5 studies investigated rituximab effects on lipids pattern.[14, 40–43] Data on other biologicals, including abatacept, anakinra, golimumab, or certolizumab have not been addressed here due to their very limited and preliminary character.

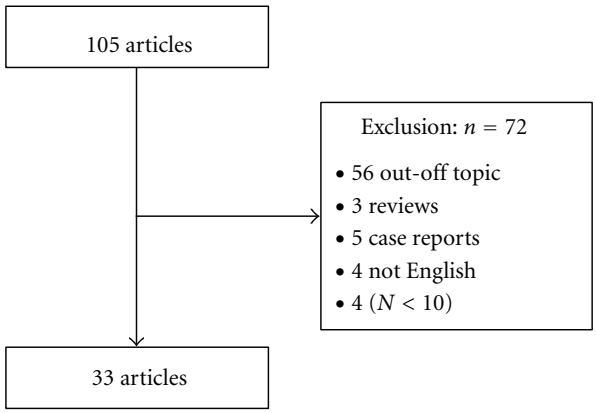

Figure 1. Flowchart

Anti-TNF Agents

TNF-α is a pro-inflammatory cytokine which plays a pivotal role in both RA and atherosclerosis pathogenesis. A beneficial effect of anti-TNF treatment on CVD morbidity and mortality in RA has been demonstrated.[44] Many studies have investigated the effects of anti-TNF medication on the lipid profile, yet the majority of studies comprise small groups of patients with a short follow-up. This paper will further focus on studies concerning infliximab, adalimumab, and etanercept. As previously mentioned, it will separately address the short and long-term effects, respectively, for all three drugs taken together. Finally, the effects on HDL-c function will be summarized.

Short-term studies

Short-term studies demonstrate primarily significant anti-atherogenic changes, particularly in TC and HDL-c levels, whereas TG and LDL-c concentrations often remain unchanged. Interestingly and of importance for our present paper, changes in the atherogenic index (TC:HDL-c) and other ratios (LDL-c:HDL-c, ApoB:ApoA-1) have also been noticed. Our group found a significant decrease of approximately 8% in both LDL-c:HDL-c and the TC:HDL-c ratio after two weeks of treatment with adalimumab in a group of 33 RA patients as compared to placebo.[24] Our results have been further confirmed by a recent study in 50 RA patients receiving adalimumab: AI baseline—16

weeks was 3.33 (0.93) versus 3.15 (0.85), P = 0.034.[32] A significant decrease in the ApoB:ApoA-1 ratio has been also reported (P = 0.014). A trend towards a more pronounced effect on HDL-c in the responders group has been noticed together with an association with disease activity changes (r = −0.31, P = 0.03). Similar results have been reported by Jamnitski et al., who found a significant decrease in the ApoB:ApoA-1 ratio over a period of 3 months [19] in 292 RA patients receiving TNF blockade. Interestingly, this change has been found only in good and moderate EULAR responders. Nevertheless, some further studies reported opposite results (Table 1). Following 45 RA patients treated with infliximab during a period of almost 6 months, our group reported a significant increase in the TC:HDL-c ratio [11] at the end of this period. These findings were supported by Dahlqvist et al.[17], who reported an increase of 8% and 9% in the LDL-c:HDL-c and TC:HDL-c ratio, over the same time period in 52 RA patients treated with infliximab. Other studies did not indicate any change in the AI or other ratios within a period of 3 or 6 months of anti-TNF therapy,[14, 16, 18, 21, 25, 26, 28, 30, 31] although individual lipid levels were often found to increase in the initial months of treatment.[25, 26, 30, 31] Studying 56 patients with RA receiving infliximab for 30 weeks, Allanore et al. found no changes in the AI despite a significant stable increase of HDL-c and TC. They also noticed no relations between response to therapy and lipid pattern modifications.[15] Similar findings have been reported by Seriolo et al. in 34 consecutive RA patients treated with various TNF blockers (n = 16 for etanercept, n = 14 for infliximab, and n = 4 for adalimumab) for 24 weeks.[26] The authors reported however on a relation between changes in HDL-c and disease activity (DAS28) by the end of the study (r = -0.52, P < 0.01), without making any reference to response rate. These findings are in line with those from a previous study, indicating a correlation between the decrease in disease activity and the increases in HDL-c 6 weeks after therapy with infliximab has been initiated.[31] This association remained after adjusting for changes in prednisone dose, age, gender, and disease duration. Although the mean AI did not change, changes in DAS28 were significantly associated with changes in the AI in the period 0 to 2 weeks. However, this association disappeared at the end of the study period (6 weeks).

A few more studies should be mentioned, which did investigate the effects of TNF blockade on lipids pattern in RA patients, however, without entirely fulfilling our inclusion criteria. Several investigators pulled together data from patients with RA and other inflammatory conditions such as ankylosing spondylitis.[20] In this setting, they found no changes in AI after 6 months of therapy with infliximab. Other studies provided data only on individual lipids without AI or other ratios.[22, 27, 29] Finally, in an elegant study, Gonzalez-Juanatey et al. investigated endothelial function and AI in a small group (n=8) of RA patients who failed on infliximab and were now treated with adalimumab. Besides rapid improvement of endothelial function, a significant decrease of the AI was observed at week 2 (3.30 ± 0.55) and at week 12 (3.28 ± 0.48) when compared with baseline AI result (3.52 ± 0.50) (P value for both comparisons = 0.012). This was associated with a decrease in disease activity and inflammation status.[45] The apparent heterogeneity of these results may be due to several factors. Firstly, it mostly concerns small-group studies enrolling RA patients from diverse countries with a distinctive health care system and lifestyle habits, including physical activity[11, 23–25, 31, 32] and alimentation (fish-rich diet in Northern Europe, Mediterranean

diet in the Southern Europe).[1 4, 15, 17, 18, 20, 26, 28] Secondly, a difference between the anti-TNF agents may be present, leading to a more pro-atherogenic profile in the case of infliximab,[11, 17] with milder effects for adalimumab and etanercept.[19, 24, 32] Thirdly, gender may also contribute to this heterogeneity, our group reporting a more pronounced effect on lipid pattern in male RA patients. Accordingly, total cholesterol and HDL-c increased more markedly 6 months after starting infliximab ($P < 0.04$), translating into a tendency of the AI to increase.[25] Finally, the response rate and the degree of response to anti-TNF therapy is likely to impact the changes in lipid profile. Though several studies have addressed the association between changes in disease activity or inflammatory status and changes in lipids concentrations, only a few investigated the association between the latter and response according to established criteria (EULAR/ACR).[19, 25, 32] These studies suggest that the AI tends to increase more in non-responders as compared to responders,[25] or to decrease only in responders.[19, 32]

Table 1. Short-term effects of anti-TNF drugs on AI and other ratios.

Study	Drug	No of patients	Duration	Effect	
				AI	Other ratios
Popa et al.[24]	ADA	33	2 wk	L	LDL-c:HDL-c
Wijbrandts et al.[32]	ADA	8	16 wk	L	ApoB:ApoA-1
Gonzalez-Juanatey et al.[45]	ADA	8	12 wk	L	-
Kume et al.[21]	ADA/ETN	42	24 wk	N	-
Seriolo et al.[26]	ADA/ETN/IFX	34	24 wk	N	-
Soubrier et al.[28]	ADA/ETN/IFX	29	14 wk	N	ApoB:ApoA-1
Jamnitski et al.[19]	ETN	292	16 wk	L	ApoB:ApoA-1
Allanore et al.[15]	IFX	56	30 wk	N	LDL-c:HDL-c
Popa et al.[1]	IFX	45	24 wk	H	-
Dahlqvist et al.[17]	IFX	52	24 wk	H	-
Popa et al.[25]	IFX	55	24 wk	H	-
Tam et al.[30]	IFX	19	14 wk	N	LDL-c:HDL-c
Vis et al.[31]	IFX	69	6 wk	N	-
Engvall et al.[18]	IFX	40	14 wk	-	ApoB:ApoA-1
Ajeganova et al.[14]	ADA/ETN/IFX	162	24 wk	-	ApoB:ApoA-1
Curtis et al.[16]	Not specified	289	8 wk	L	-

i. ETN; etanercept, ADA; adalimumab, IFX; infliximab, AI; atherogenic index, wk; weeks, L; lower, N; neutral, H; higher

Long-Term Studies.

During the first year of treatment with anti-TNF agents, lipid concentrations tend to increase, with some reporting a return to baseline levels after an initial increase.[23] Despite a constant dosage of the anti-TNF drug, changes in AI reported by short-term studies are often not sustained over longer periods of time. Using etanercept in a group of 292 RA patients, Jamnitski et al. found a more pronounced decrease of ApoB:ApoA-1 ratio 4 months after therapy has been initiated as compared to one year time point, whereas TC:HDL-c ratio remained similar throughout study period.[19] The authors have also performed an analysis in patients who responded and patients who did not respond to the therapy according to the EULAR response criteria. There was a trend

towards a lower AI both at 4 months and at one year after starting etanercept in the responder's subgroup compared to the non-responders, reaching significance in the case of ApoB:ApoA-1 ratio (P = 0.005). Wijbrandts et al. also reported an improvement of the AI 52 weeks after adalimumab has been started in a group of 44 RA patients,[32] with ApoB:ApoA-1 ratio decreasing with 7% (P = 0.05) and TC:HDL-c ratio with 4% (P = 0.27). Of note, both ratios reached statistical significance 16 weeks after starting adalimumab (P = 0.014 and P = 0.034, resp.).[32] In contrast, in a case-control study of 52 established RA patients and 70 early RA patients, Dahlqvist et al. reported that the LDL-c:HDL-c and TC:HDL-c ratios significantly worsened one year and even two years after infliximab was started: 9.2% and 10.4%, respectively.[17] In line with this, our group found a significant increase in the TC:HDL-c ratio in a group of 55 RA patients treated with infliximab: 9% after 6 months (P = 0.02) and 4% after 12 months (P = 0.05).[25] In the same study, LDL-c:HDL-c ratio did not significantly changed over time. Peters et al. found no change in the ApoB:ApoA-1 ratio and TC:HDL-c ratio, respectively, in a group of 80 RA patients treated with infliximab for a period of 48 weeks.[23] Interestingly, they observed that changes in prednisone dose were related to changes in HDL-c and TC, with a relatively greater impact on HDL-c, resulting in an inverse association between prednisone dose and AI (TC:HDL-c and ApoB:ApoA-1).[23] Finally, in a large study involving different anti-TNF agents (infliximab, adalimumab, and etanercept), Ajeganova et al. found no changes in ApoB:ApoA-1 ratio in all three subgroups according to the drug, 12 months after therapy has been initiated.[14] Similar results have been previously reported by Engvall et al., who observed no change in ApoB:ApoA-1 ratio between 3 months and 2 years of follow-up.[18] Both studies report no data on TC:HDL-c index. Despite apparent discrepancy, some trends maybe depicted when analyzing these long-term effects of anti-TNF drugs on lipids in patients with RA. These trends become clearer when focusing on AI, which demonstrates therefore to be superior to individual lipid concentrations in this respect (Table 2). Therapy with etanercept or adalimumab seems to have a positive impact on AI, although this improvement does not always reach statistical significance.[19, 32] In contrast, the use of infliximab may worsen lipid ratios on the long term,[17, 25] though some report a neutral effect.[23] Nevertheless, a rapid and sustained control of disease activity as in the case of responders would be associated with better ratios compared to non-responders, even in those patients treated with infliximab.[23] Alternatively, the concomitant use of prednisone may influence AI. Given the prognostic value of these ratios for future CVD, it is likely that these changes are clinically relevant and may contribute to the decreased incidence of myocardial infarction and other CVD events observed with anti-TNFα treatment in RA.

Anti-TNF therapy and HDL-c function

The link between HDL-c and cardiovascular disease risk is far more complex than originally thought. This may be explained by the inherent heterogeneity of HDL-c particles in terms of composition, structure, and biological function. Emerging evidence suggests that for instance small dense protein-rich HDL3-c particles are less capable of protecting LDL against oxidative modification.[46] This has led some to propose that the functionality of HDL-c may be as relevant as plasma levels of HDL-c to CVD risk assessment.[47, 48] In the same context, a number of studies have demonstrated that inflammation is able to negatively impact the anti-atherogenic

properties of HDL-c.[49] The issue becomes of interest thus in the case of patients suffering from chronic inflammatory diseases, such as RA. In a study on 48 RA patients, which also included patients with SLE and healthy controls, McMahon et al. demonstrated for the first time the presence of a pro-inflammatory HDL-c in this group of patients.[10] About 20% of RA patients were likely to have such an HDL-c, as compared to 4% of healthy controls. HDL-c function tended to correlate with ox-LDL concentrations (r = 0.355). Inflammatory markers and prednisone dosage have been shown to be associated with a pro-inflammatory HDL-. Interestingly, the authors found no association between HDL-c function (pro-inflammatory) and HDL-c concentrations, an observation which has been recently confirmed by an elegant study in the general population.[47] Statins may reverse the pro-inflammatory HDL-c as was observed in a small group of RA patients during a period of 12 weeks.[50] This improvement was not entirely contributed to a decrease in inflammatory state. It was further indicated that the pro-inflammatory function of HDL-c in RA might be due to a different composition as compared with anti-inflammatory HDL-c,(51) including a lower LCAT activity and higher MPO activity. Nevertheless, the study does not provide sufficient evidence to support the standard use of statins in patients with RA. Our group has investigated for the first time the effects of anti-TNF therapy on HDL-c anti-atherogenic function. We found that infliximab is able to improve HDL-c anti-oxidative capacity, an effect that was sustained 6 months after anti-TNF therapy has been initiated.[11] It is still unclear how stable these effects are further in the course of therapy and whether they are solely due to TNF blockade or more likely to reflect the overall inflammatory suppression achieved in these patients. Recently, we observed that HDL-c subfractions are modified in RA patients, especially in women,[51] reinforcing again the importance and in the same time the complexity of HDL-c status in these patients with respect to their CVD risk. Whether anti-TNF drugs are able to restore this detrimental HDL-c profile remains a subject for further investigations.

Table 2. Long-term effects of anti-TNF drugs on AI and other ratios.

Study	Drug	No of patients	Duration	Effect	
				AI	Other ratios
Jamnitski et al.[19]	ETN	292	1 year	N	ApoB:ApoA-1
Wijbrandts et al.[32]	ADA	50	1 year	N	ApoB:ApoA-1
Dahlqvist et al.[17]	IFX	51	2 years	H	-
Popa et al.[25]	IFX	55	1 year	H	LDL-c:HDL-c
Peters et al.[23]	IFX	80	1 year	N	ApoB:ApoA-1
Ajeganova et al[14]	ETN/ADA/IFX	162	1 year	-	ApoB:ApoA-1
Engvall et al.[18]	IFX	18	2 years	-	ApoB:ApoA-1

i. ETN; etanercept, ADA; adalimumab, IFX; infliximab, AI; atherogenic index, N; neutral, H; higher

Anti-IL6 agents

Interleukin (IL6) is another cytokine that plays a key role in the pathogenesis of chronic inflammatory diseases. Recently, the therapeutic blockade of its receptor proved to efficiently suppress disease activity in patients with RA.[33–35, 37, 39] Owing to the increased CVD risk and anti-TNF experience, trials investigating the effects of the IL-6 receptor (IL-6R) antagonist

tocilizumab (TCZ) in patients with RA have included for the first time the impact of the therapy on the lipid pattern as part of the safety analysis of the drug. An increase of individual lipid concentrations has been constantly reported with TCZ.[37, 38] Nevertheless, detailed results regarding the effect of treatment on the AI could not be derived from all of the studies (Table 3). Maini et al. reported that lipids levels increased initially and then stabilized and did not continue to increase during the treatment period, which is comparable to the effects reported in anti-TNF studies. Importantly, the mean AI remained largely unchanged and was reduced to below its initial level by the 20-week follow-up visit in the groups receiving 8 mg/kg of TCZ.[37] In another trial by Emery et al., 20-week therapy with TCZ resulted in higher rate of more than 30% increase in LDL-c:HDL-c ratio in patients receiving the drug compared to controls: 22.2% (TCZ 8 mg/kg), 19.1%(TCZ4 mg/kg), and 10,1% (controls), respectively.[33] In contrast, comparable proportions of patients had greater than 30% increase in the ApoB:ApoA-1 ratio: 11.6% (TCZ 8 mg/kg), 9.4% (TCZ 4 mg/kg), and 9.7% (controls), respectively. No acute CVD event has been reported during the study period. In the OPTION study comparing two TCZ regimens with placebo, Smolen et al. report similar results.[39] Increases in the TC:HDL-c ratio of more than 30% above baseline were observed in 17% of patients treated with TCZ 8 mg/kg, 8% of patients receiving TCZ4 mg/kg and 5% in the placebo group. A comparable ApoB:ApoA-1 ratio between the groups have been reported however. One last trial adds to strengthen the previous presented data (TOWARD study).[34] In this study patients receiving TCZ 8mg/kg and a DMARD were compared to patients receiving a DMARD and placebo. The authors indicate increases of more than 30% in the TC:HDL-c ratio in 12% and 7% of patients in the TCZ and control group, respectively, and increases of more than 30% in the LDL-c:HDL-c ratio in 20% and 12% of patients, respectively. Again, no significant changes in the ApoB:ApoA-1 ratio have been noticed in both groups. Finally, Jones et al. compared the monotherapy with TCZ and methotrexate in a group of 673 RA patients (AMBITION study).[35] They report no data on AI during the 24 weeks of therapy. It was however noted that TCZ is more prone to disturb lipid pattern compared to methotrexate and leads to LDL-c and TG elevations. In an observational study, Kawashiri et al. noticed no changes in the ApoB:ApoA-1 and TC:HDL-c ratio despite an increase of individual lipids in a small group of RA patients treated with TCZ for 12 weeks.[36] Similar findings have been reported by Kume et al., who found no changes in TC:HDL-c ratio 24 weeks after starting tocilizumab in 22 RA patients, despite sustained increase of both TC and HDL-c alone.[21] Interestingly, the authors noticed that the increase in TC in the TCZ group has been higher than in the patients receiving adalimumab or etanercept, reaching statistical significance (TCZ versus ETN $P = 0.024$, TCZ versus ADA $P = 0.032$). Although the first of its kind by directly comparing three different biologicals with respect to endothelial dysfunction and lipid pattern, the results of the study should be interpreted with caution given the relative low number of patients enrolled in each group (approximately 20). Overall, the present experience with tocilizumab appears to suggest a certain detrimental effect on lipids pattern, translated into a higher percentage of patients with a significant increase in the AI—TC:HDL-c and LDL-c:HDL-c,[33, 34, 39] whereas ApoB:ApoA-1 ratio remains stable throughout the therapy.[33, 36, 39] These lipid modifications led in several cases to the start of therapy with lipid-lowering agents. It is still unclear if long-term treatment with TCZ would reverse these detrimental effects and achieve sustained improvements in AI. To our knowledge, no studies have investigated the

effect of TCZ on the HDL-c cholesterol function. Given the emerging importance of this factor in CVD risk assessment, future studies on this issue are warranted.

Table 3. Effects of tocilizumab on atherogenic index and other lipid ratios.

Atherogenic index	Study [reference], patients (n), lipid ratio's
Higher	Emery et al.[33], (n = 338), LDL-c:HDL-c
	Genovese et al.[34], (n = 803), TC:HDL-c, LDL-c:HDL-c
	Smolen et al.[39], (n = 418), TC:HDL-c
Neutral	Kume et al.[21], (n = 22), TC:HDL-c
	Kawashiri et al.[36], (n = 19), TC:HDL-c, ApoB:ApoA-1
	Maini et al.[37], (n > 50), TC:HDL-c
Not assessed	Jones et al.[35], Schultz et al.[38]

Rituximab

There were few studies investigating the effects of newer biologicals on lipids pattern in RA patients. Rituximab, a B-cell depletion drug, targeting the CD20 positive B lymphocytes, has been so far been scarcely investigated with regards to its effects on AI and HDL-c composition. In a small group of RA patients, Gonzalez-Juanatey et al. investigated for the first time the effects of rituximab on lipid parameters.[40]. The authors have found a slight, nonsignificant increase in HDL-c levels both at 2 weeks (56 ± 11 mg/dl) and at 6 months (57 ± 15 mg/dl) compared to baseline levels (52 ± 11 mg/dl), whereas TC increased only 2 weeks after starting rituximab (211± 42 mg/dl versus 191 ± 37 mg/dl). No direct information on AI has been provided. In another study, Kerekes et al. found an increase in HDL-c levels with 14.3%, 33.1%, and 35.4% as compared to baseline, at 2, 6, and 16 weeks, respectively, after rituximab had been initiated.[41] At sixteen-week time-point, the difference reached significance (P = 0.035). Interestingly, TC tended to decrease without significance, throughout study period. This may suggest a decrease in the AI. The results are in line with the previous ones, yet the limited number of patients investigated (n = 5) makes their interpretation difficult. The first larger study comes from Ajeganova et al.[14] The Swedish group investigated the effects of various biologicals on lipids pattern in 215 RA patients receiving therapy with various biologicals, focusing on apolipoproteins (ApoA and ApoB) and their ratio. The investigators found that in the rituximab-treated group (n = 53) apoA-1 levels increased throughout the study with 0.09 ± 0.32 g/L (P =0.022, follow-up of 6 months) and 0.09 ± 0.32 g/L (P=0.06, follow-up of 12 months), respectively. The ratio ApoB:ApoA-1 remained relatively stable and did not change significantly over the study period. The TC, HDL-c, and their ratio (AI) have been not assessed. Interestingly, the authors found no associations between ApoB:ApoA-1 ratios and markers of disease activity, therefore sustaining our hypothesis that ratios are less susceptible to changes in disease activity and thus they are probably better suited to predict CVD risk in these patients. Finally, two more studies should be mentioned, which further investigated the interplay between rituximab and lipids in RA patients by assessing the effects of this drug on HDL-c anti-atherogenic function.[42, 43] In the first one, 49 RA patients have been followed 6 months after receiving rituximab.[43] As previously suggested, rituximab modestly increased HDL-c and ApoA-1 levels and significantly improved AI($P < 0.05$). A subanalysis

revealed that these changes were only present in the subgroup of responders. There is no association found with the use of prednisone. HDL-c composition changed upon rituximab therapy, becoming depleted in SAA-1 in patients who have demonstrated a good response to the therapy, rendering the molecule to be anti-atherogenic. This observation further substantiates the importance of HDL-c function assessment in patients with RA and other chronic inflammatory conditions in order to get a proper picture of their CV risk. In the second study, Mathieu et al. presented data on 33 RA patients treated with rituximab.[42] AI remained stable, although TC significantly increased both at 6 and at 12 months after rituximab ($P < 0.001$). The study enrolled RA patients with longer disease duration (mean 17.6 years) who had already failed to respond to two different types of anti-TNF drugs.

CONCLUSION

The available literature shows that anti-TNF drugs, IL-6R antagonists, and anti-CD20 antibodies are able to modulate the lipid profile in RA. Interestingly, when considering their effects on the AI and other lipoproteins ratio, it becomes evident that changes in individual lipid levels often do not translate in to a change in AI, or are not sustained long enough to significantly affect the AI. Therapy with etanercept, adalimumab, or rituximab seems to have a positive impact on AI, although this improvement does not always reach statistical significance and sometimes an initial gain is lost over time. In contrast, the use of infliximab may worsen lipid ratios on the long term, though some report a neutral effect. Similarly, tocilizumab is likely to worsen lipid ratios in the first months after therapy has been initiated, while the longer-term effects remain still unknown. Nevertheless, controlling disease activity and achieving remission seem to beneficially impact the lipid pattern, as suggested by the positive effects seen in responders. Finally, the form and function of HDL-c appear to be compliant to changes in inflammation. Treatment with anti-TNF agents and rituximab results in improvements of the HDL-c anti-atherogenic capacity. It is unclear whether these changes progress over time and to what extent they decrease CVD risk. No data on the effects of tocilizumab on HDL-c function are available. The interpretation of our conclusions should not be without caution. It is still unclear to what extent these changes actually lead to a change in the CVD risk. Moreover, some suggest that even if changes occur, they might have a milder impact on CVD risk compared to the general population.[52] The follow-up period of these studies is often too short to include CVD events. Sometimes possible confounding variables were not properly accounted for as changes in lipid levels often were not a primary outcome, for instance in the majority of tocilizumab studies.

In conclusion, we suggest that AI and HDL-c function are more suitable parameters of lipid profile as determinants of CVD risk in patients with RA, and perhaps for other chronic inflammatory diseases including lupus, psoriatic arthritis, and ankylosing spondylitis. The effects of biologicals on these parameters depend on the response rate, concomitant prednisone use, duration of therapy, and the biological self. If CVD risk management becomes an integrated part of therapeutic strategies in RA and given the increasing importance of personalized medicine, the

choice of biological might become partially dependent on the impact on CVD risk profile. Future studies with clinical CVD endpoints would have to address the value of monitoring AI and HDL-c function during therapy with biologicals in order to establish their real impact on CVD risk in these patients.

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CHAPTER 6

The effect of disease duration and disease activity on the risk of cardiovascular disease in rheumatoid arthritis patients

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Annals of Rheumatic Diseases
2015; 74: p 998-1003

ABSTRACT

Objective

Disease duration and disease activity may be associated with an increased risk of cardiovascular disease (CVD) in rheumatoid arthritis (RA). The objectives of this study were to investigate (1) the relationship between duration of inflammation and the development of CVD and (2) the relationship between RA disease activity over time and CVD in patients with RA.

Methods

RA patients with a follow-up of ≥6 months in the Nijmegen early RA cohort without prior CVD were included. Disease activity over time was calculated using the time-averaged 28 joint disease activity score (DAS28) for each patient. Kaplan–Meier survival analysis and Cox proportional hazards regression were used for the analyses.

Results

During follow-up of the 855 patients that were included, 154 CV events occurred. The course of hazards over time did not indicate a change in the risk of CVD over the course of RA (disease duration), which is also reflected by the absence of a deflection in the survival curves. The survival distributions did not differ between patients with a disease duration of <10 years or >10 years (Log-rank test: $p = 0.82$). Time-averaged DAS28 was significantly associated with CVD ($p = 0.002$) after correction for confounders.

Conclusions

Disease duration does not appear to independently affect the risk of CVD. The risk of CVD in RA patients was not increased after 10 years of disease duration compared with the first 10 years. Disease activity over time may contribute to the risk of CVD.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of multifactorial aetiology. Despite important advances in treatment strategies, RA is still associated with excess mortality rates of approximately 25%.[1] Cardiovascular disease (CVD) represents the leading cause of death in RA, accounting for approximately 50% of all excess mortality.[2] The excess risk of CVD cannot be completely explained by traditional risk factors alone.[3] Growing evidence supports the notion that inflammatory and immune mechanisms underline atherosclerosis.[4, 5] It is hypothesized that chronic systemic inflammation in RA represents a disease related risk factor, accounting for extra cardiovascular (CV) risk.[6] Recent studies have investigated the link between the presence of inflammatory markers used to determine disease activity in RA, such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and the development of CVD. CRP has been indicated as a predictor for (accelerated) atherosclerosis.[7, 8] ESR and CRP have also been associated with CVD in RA and polyarthritis.[9–12] Atherosclerotic plaques in the carotid artery appear more severe and prevalent in RA patients compared with the general population.[13–16] In comparison to healthy controls and RA patients in remission, RA patients with active disease seem to have less stable plaques, more vulnerable to rupture, increasing the probability of an acute CVD event.[17] Additionally, inflammation is likely to modulate traditional CVD risk factors, including lipids, endothelial function and insulin sensitivity.[18, 19] This led to the 'smaller slice of a larger pie' concept in which traditional CVD risk factors have a smaller contribution to CVD risk in RA patients than in the general population.[6] The genotype of RA patients may also be of interest, potentially contributing to unfavourable lipid patterns and accelerated atherosclerosis.[20, 21] Although a growing body of pathophysiological evidence supports the hypothesis that inflammation contributes to the development of CVD, clinical research has yet to clearly demonstrate the relationship between RA disease activity and CVD. In addition to disease activity, perhaps the time spent by a patient in an inflammatory state would be crucial for the chance to develop CVD. Long-standing disease elongates a patient's exposure to chronic inflammation, and it has been suggested that this prolonged exposure has a cumulative effect and disease duration may therefore act as a separate CVD risk factor.[22] Disease duration over 10 years has been indicated as a risk factor for CVD in the European League Against Rheumatism (EULAR) recommendations for CVD risk management in RA patients,[23] even though some studies have shown that the risk of CVD is already increased in the early, sometimes preclinical stages of RA compared with the general population.[24–26] However, the excess risk of CVD reported in early RA may still continue to increase as the disease progresses over time. Therefore, the objectives of the present study are (1) to investigate the relationship between duration of inflammation and CVD risk corrected for the level of inflammation and (2) to investigate the relationship between RA disease activity and CV risk in patients with RA.

METHODS

Study design and patients

This study is based on mainly prospectively collected data from the Nijmegen early RA inception cohort. Patients were included at diagnosis of RA (baseline) in the outpatient clinic of the departments of rheumatology of the Radboud University Medical Centre (since 1985) or the Maartenskliniek (since 1990) in Nijmegen, The Netherlands. At inclusion, patients had a disease duration of <1 year, were disease-modifying anti-rheumatic drug (DMARD)-naive and fulfilled the 1987 ACR criteria for the classification of RA. All patients provided written informed consent, and this project was approved by the medical ethical committee, CMO Arnhem Nijmegen. Patients were included if follow-up time was >6 months and if they had no history of CVD prior to RA diagnosis. The total cohort included 1018 patients at the time of analysis. The 77 patients who had a history of CVD prior to inclusion were excluded, as well as 86 patients who had a follow-up of less than 6 months. In total, 855 patients were included for analysis.

Primary and secondary outcomes

The primary outcome in this study was the occurrence of a first CVD event by physician diagnosis, which was retrieved by extensive review of medical charts and electronic patient files. Included events were acute coronary syndrome comprising both myocardial infarction (MI) and unstable angina pectoris, angina pectoris, cerebrovascular event or stroke, transient ischemic attack (TIA), peripheral artery disease (PAD) and revascularisation procedures including coronary artery bypass surgery, percutaneous coronary intervention and percutaneous transluminal coronary angioplasty. Both fatal and non-fatal events were included. Deaths due to CVD were verified from death certificates, provided by Statistics Netherlands.[27] Heart failure, cerebral haemorrhage and non-coronary cardiac death (i.e., arrhythmias) were not included for the purpose of this study.

Assessments

Baseline patient characteristics were retrieved from the RA inception cohort database, including age, sex, rheumatoid factor (RF) status, disease activity (28 joint disease activity score (DAS28)), initial anti-rheumatic treatment and treatment with biological DMARDs. The time-averaged DAS28 score was calculated by taking the area under the curve of the DAS28 score of the total follow-up period divided by the follow-up period. Baseline data regarding CVD risk factors were collected by review of patients' charts and electronic patient files, including smoking status (yes/no (Y/N)), blood pressure, use of medication preventative for CVD, body mass index (BMI) and diabetes mellitus. Non-fasting blood samples were used to measure total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c).

Statistical analysis

The cut-off point of 10 years was used to differentiate between patients with and without 'long-standing' disease, in reference to the EULAR recommendations that indicate disease duration >10 years as a risk factor for CVD. Differences in baseline variables between patients who had a CVD before 10 years of disease duration and patients who developed CVD after 10 years were tested

using independent samples t-test, Wilcoxon test or $\chi 2$ test, as appropriate. The effect of both disease duration and disease activity on the risk of CVD was analysed by Kaplan-Meier survival analysis and Cox proportional hazards regression analysis. Survival and hazard curves were used to visualise the risk of CVD over time and estimate the effect of disease duration on CVD risk. Sex, age, smoking (Y/N), BMI (weight (kg)/height(m)^2), systolic blood pressure (mm Hg), TC (mmol/L), HDL-c (mmol/L), DAS28, ESR (mm/h), CRP (mg/L), swollen and tender joints, visual analogue scale (VAS, mm) and diabetes at baseline (Y/N), treatment for CVD risk factors (Y/N), initial anti-rheumatic treatment (methotrexate, sulfasalazine or other), (MTX) ever (Y/N), treatment with biological ever (Y/N) and RF status (positive/negative) were considered as possible confounders. The risk of a CVD event during the first 10 years of the disease was compared with the risk of a CVD event after 10 years of disease duration (up to 25 years) by means of log-rank testing and Kaplan-Meier survival analysis. For this purpose, survival experience was grouped into two. For the first group (group 1) patients were selected who were at risk for a first CVD event in the first 10 years of disease duration, that is, all patients who were included in this study. Patient time stopped at the time of event, or was censored after at the latest after 10 years of event-free follow-up. For the second group (group 2), patients were selected (again) if they had a follow-up >10 years and were free of CVD up until that point. Patient time stopped at the time of event or was censored at the latest at the censoring date 30-09-2011. The effect of the level of inflammation over time (time-averaged DAS28 and time-averaged ESR) was analysed using Cox proportional hazard regression with time-averaged DAS28 as the main independent variable. Age, sex, smoking (Y/N), BMI (weight (kg)/height(m)2), systolic blood pressure (mm Hg), TC (mmol/L), HDL-c (mmol/L), DAS28 and diabetes at baseline (Y/N), RF status (positive/negative) and initial anti-rheumatic treatment (methotrexate, sulfasalazine or other), treatment with methotrexate ever (Y/N), treatment with biological ever (Y/N) were considered as possible confounders. Missing values on variables were imputed using multiple imputation analysis with five repetitions.

RESULTS

Patient characteristics and CV events

In total, 855 patients were included, comprising 9959 patient years. Patients had a mean±SD disease duration of 11.7±6.1 years. Patient characteristics at baseline are presented in table 1 for the whole group and separately for patients with a CVD event within the first 10 years following disease onset and with a CVD event after more than 10 years. In group 1, 76% of patients were treated with MTX compared with 73% in group 2, and 30% were treated with a biological in group 1 versus 33% in group 2. A total of 154 CVD events, of which 16 were fatal, were registered, including 64 cases of acute coronary syndrome, 19 cases of stable angina pectoris, 30 cases of strokes, 15 cases of TIAs, 21 cases of PAD and 5 revascularisation procedures. Missing values ranged from 0.1% for RF to 10.3% for smoking at baseline.

Disease duration and the risk of CVD

The linearity of both the survival and the proportional hazard curve shows that the cumulative risk increased with a similar rate as disease duration increased and that the risk per year remained constant (figure 1A); also, the hazard for CVD remained similar as disease duration increased (figure 1B).

Table 1. Patient characteristics

	All (n=855)	Event <10 yrs (n=113)	Event ≥ 10yrs (n = 41)	p-value
Age (years), mean±SD	54±13.8	62±9.7	55±8.7	**<.001**
Sex (female), N(%)	571 (67)	63 (56)	23 (56)	0.97
DAS28	5.0±1.3	5.4±1.3	5.5±1.3	0.83
SJC, median (P25-P75)	9 (5–13)	10 (6–15)	11 (6–15)	0.60
TJC, median (P25-P75)	6 (2–11)	8 (3–13)	8 (3–13)	0.97
ESR (mm/h), median (P25-P75)	30 (16–49)	35 (18–48)	39 (15–60)	0.45
VAS (mm), median (P25-P75)	46 (27–59)	50 (31–65)	47 (31–60)	0.37
Rheumatoid factor (positivity), N(%)	664 (78)	93(82)	35 (85)	0.65
Initial anti-rheumatic treatment				
Methotrexate, N(%)	135 (16)	28 (25)	3 (7)	**<.001**
Sulfasalazine, N(%)	525 (61)	69 (61)	31 (76)	**0.09**
Other, N(%)	195 (23)	29 (26)	7 (17)	0.27
Smoking, N(%)	256 (30)	45 (40)	16 (39)	0.93
BMI (weight[kg]/height[m]^2), mean±SD	23.7±6.8	27±4.0	25±3.3	0.10
Diabetes, N(%)	29 (3.4)	11 (10)	1 (2)	0.14
SBP (mmHg), mean±SD	145±25	155±24	148±20	0.10
TC (mmol/L), mean±SD	5.9±2.4	5.2±1.4	5.5±1.4	0.27
HDL-c (mmol/L), mean±SD	1.5±0.6	1.2±0.3	1.4±0.4	**<.001**
TC:HDL-c ratio (mmol/L), mean±SD	4.2±0.9	4.5±1.2	4.0±0.8	**0.01**
CVD prevention, N(%)†	129 (15.1)	30 (27)	11 (27)	0.97

i. P-value for the difference between RA patients with a CVD event before and after 10 years of disease duration.

ii. †CVD prevention represents medication use for primary prevention of CVD, including anticoagulants, ACE or angiotensin II inhibitors, β-blockers, diuretics, calcium antagonists and statins or fibrates.

iii. DAS28; 28-joint disease activity score, SJC; swollen joint count (number of swollen joints out of 28), TJC; tender joint count (number of tender joints out of 28), ESR; erythrocyte sedimentation rate, VAS; visual analogue scale, BMI; body mass index, SBP; systolic blood pressure, TC; total cholesterol, HDL-c; high-density lipoprotein cholesterol, CVD; cardiovascular disease.

If the risk of CVD would increase as disease duration increases, the cumulative survival and (expected) hazard lines would show a curve upwards as time progresses. After correction for confounders (table 2), the curves did not change (not shown). The results from the Kaplan-Meier survival analysis in which the risk of CVD during the first 10 years of disease duration (group 1) was compared with the risk of CVD after 10 years of disease duration (group 2) show similar survival distributions that did not differ significantly between groups, with p=0.82. The survival distributions are presented in figure 2, showing overlapping curves.

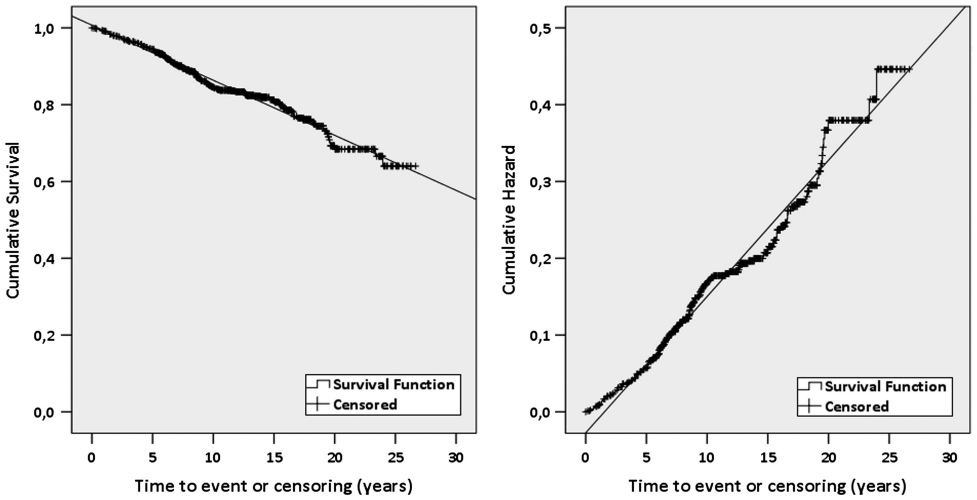

Figure 1. Disease duration until CVD event or censoring in rheumatoid arthritis patients from the 1985 Nijmegen inception cohort. Cumulative survival of cardiovascular disease (CVD) or event-free patients and the cumulative hazard are depicted on the y-axis of panels (A) and (B), respectively. Time to event or censoring (disease duration) is depicted on the x-axis. As disease duration increases, the relative increase in CVD risk remains similar, resulting in a linear survival (A) and hazard (B) curve

Table 2. Effect of disease duration on the risk of cardiovascular disease; results of the Cox proportional hazards model with disease duration until event or censoring as the time variable

	Estimate	SE	Exp(B)	p-value	95% CI	
					Lower	Upper
Age (years)	0.049	0.008	1.051	<.001	1.035	1.067
Smoking (Y/N)	0.424	0.174	1.528	0.015	1.087	2.149
BMI (weight[kg]/height[m]^2)	0.065	0.018	1.067	<.001	1.030	1.105
HDL-c (mmol/L)	−.664	0.211	0.515	0.002	0.340	0.912
Diabetes (Y/N)	1.050	0.312	2.857	0.001	1.549	5.268
Time-averaged DAS28	0.430	0.081	1.537	<.001	1.312	1.802
Bioloigical ever (Y/N)	−.538	0.227	0.584	0.018	0.374	0.912
MTX ever	−.336	0.187	0.715	0.072	0.496	1.031

i. Cox proportional hazards regression model with disease duration until event or censoring as the time variable. Corrected for confounders.

ii. BMI; body mass index, DAS28; 28-joint disease activity score, HDL-c; high-density lipoprotein cholesterol, MTX; methotrexate.

Disease activity and the risk of CVD

The mean±SD time-averaged DAS28 was 3.6±1.1. The results of the Cox proportional hazard regression with the time-averaged DAS28 as the main independent variable are presented in table 3. After correction for confounders, the time-averaged DAS28 had a significant effect on the risk of CVD (p=0.002). With every point the DAS28 increases, the hazard for CVD increases with 0.281. Overall there was a difference in survival distributions between patients with low (<3.2),

moderate (3.2–5.1) and high (>5.1) DAS28 over time (p=0.028). The survival curve of patients with a consistently high disease activity, that is, a time-averaged DAS28 >5.1, was the lowest (figure 3). After correction for confounders, this group (DAS28 >5.1) did not have a significantly different effect on CVD risk compared with the <3.2 group (p=0.074). Time-averaged ESR was not significantly associated with CVD (p=0.805) (data not shown).

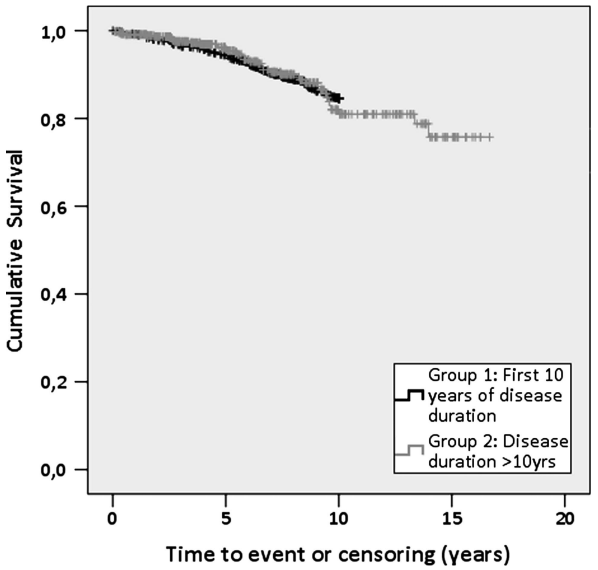

Figure 2. Disease Survival distribution for the group of patients at risk for cardiovascular disease (CVD) before 10 years of disease duration (group 1) and patients still at risk for CVD after 10 years (group 2). Cumulative survival of CVD is depicted on the y-axis, and time to a CVD event or censoring (disease duration) is depicted on the x-axis.

Table 3. Effect of disease activity on the risk of cardiovascular

	Estimate	SE	Exp(B)	p-value	95% CI	
					Lower	Upper
Crude model						
Time-averaged DAS28	**0.271**	0.073	1.311	<.001	1.135	1.514
Adjusted model						
Time-averaged DAS28	**0.281**	0.092	1.325	0.002	1.106	1.588
Sex (male)	**0.675**	0.173	1.964	<.001	1.399	2.759
Age (years)	**0.047**	0.008	1.048	<.001	1.033	1.065
BMI (weight[kg]/height[m]^2)	**0.090**	0.013	1.094	<.001	1.066	1.123
RF (positive)	**0.300**	0.224	1.349	0.182	0.869	2.095
DAS28 at baseline	**0.114**	0.073	1.120	0.118	0.971	1.292
Bioloigical ever (Y/N)	**−.609**	0.217	0.544	0.005	0.355	0.832

i. Results shown of the Cox proportional hazards regression analysis for the crude model without correction for confounders (crude model) and for the adjusted model after correction for confounders.

ii. BMI; body mass index, DAS28; 28-joint disease activity score, RF; rheumatoid factor, MTX; methotrexate.

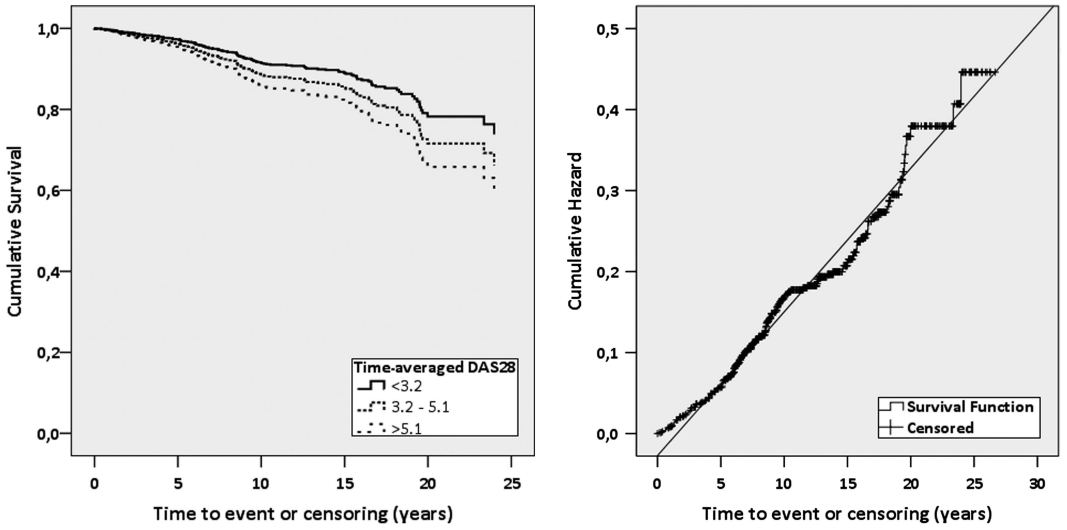

Figure 3. Survival distributions for rheumatoid arthritis patients divided into three groups based on the time-averaged DAS28 (<3.2, 3.2–5.1, >5.1). Cumulative survival of cardiovascular disease (CVD) is depicted on the y-axis, and time to a CV event or censoring is depicted on the x-axis

DISCUSSION

According to the results of this study, disease duration did not appear to affect the risk of CVD in patients with RA. Furthermore, our data showed that mean RA disease activity over the course of the disease (time-average DAS28) may contribute to the risk of CVD. The survival distribution for CVD was linear as disease duration increased. Specifically, the shape (linearity) of the survival curves during the first 10 years of RA and during the years thereafter was very similar, and the survival distributions were not significantly different. If CVD risk would increase in patients with long-standing disease, the survival distribution curve would be expected to bend upwards as time progresses. The EULAR recommendations include disease duration >10 years as a risk factor for CVD, but the evidence for this choice is limited.[23] In a study by Gabriel et al.[1] in which survival trends in the RA population were investigated, it was reported that the excess mortality did not become apparent until 8–10 years after disease onset,[1] that is, after longer disease duration. However, this association was observed for all-cause mortality and the effect of disease duration on the risk of CVD was not investigated separately in this study. In a study by Naz et al,[28[it was reported that that all-cause and CVD mortality was increased in RF-positive RA patients compared with the general population. CVD mortality was increased in both early (5 years) and late follow-up (10 years). This is more in line with findings from several other studies that suggest the excess risk of CVD is already present in the early stages of RA.[24–26] In our cohort, a higher prevalence of CVD was found in patients during their first 10 years of RA compared with patients still at risk for their first CV event after 10 years. Pathophysiological data have delivered support for the

notion of disease duration as risk factor for CVD, as it has been reported that (accelerated) atherosclerosis appears to be more severe in RA patients with established disease.[22] However, this cross-sectional association with a surrogate marker has not been shown to translate in increased risk for actual CVD. Also, patients with RA appear to be more prone to plaque instability and rupture, in addition to accelerated atherosclerosis. Inflammation in RA may therefore contribute more specifically to more severe acute coronary syndromes and strokes,[29] which may be more strongly associated with the presence and severity of local or systemic inflammation than with disease duration. The results of this study suggest that increased disease activity over time increases CVD risk. Interestingly, ESR did not appear to significantly affect CVD risk. Additional research is necessary to further investigate the relationship between individual DAS28 components and CVD risk. Different patient groups divided based on disease activity (<3.2, 3.2–5.1, >5.1) did not differ significantly from each other in terms of association with CVD risk. However, when looking at the differences in the survival curves, patients with a consistently high level of disease activity (time-averaged DAS28>5.1) appear to be at a significantly higher risk of developing CVD compared with patients with lower disease activity levels. In a previous study, we have suggested that an increased risk of CVD may already be observed in patients with low levels of disease activity[30] as we have shown that there was no difference in the level of disease activity or the number of patients with low to moderate levels of disease activity between RA patients with and without MI (and similar disease duration).[30] This may also mean that complete sustained eradication of systemic inflammation, that is, sustained clinical remission, is required to substantially reduce CVD risk in RA patients. Indeed, there is some evidence to suggest that remission may be beneficial in preventing CVD events.[31, 32]

There are some points that should be considered when interpreting the results of this study. The cohort that was used in this study spans more than 25 years and provides a relatively large sample of RA patients with long follow-up times and a considerable number of recorded CVD events, which increases the reliability of our findings. However, calendar time trends in the prevalence of CVD may be a source of bias. In the general population in both USA and Western Europe,[33–35] the risk of CVD has been reduced in the past decades, while in RA the risk of CVD does not appear to have changed.[1] Therefore, a calendar time trend on the prevalence of CVD in our cohort was considered unlikely. Also, medication used to treat the systemic inflammation in RA, particularly the recent introduction of biological DMARDs, may affect CVD risk. Initial anti-rheumatic treatment and treatment with MTX or a biological during follow-up were included in the analysis and proved to be confounders. The widespread introduction of biologicals may lead to an overall decrease in disease activity levels in the RA population and to more effective suppression of systemic inflammation in individual RA patients. We did not investigate the effect of specific dosages of anti-rheumatic medication or cumulative (biologic) DMARD use. The possible beneficial effects of more recently developed anti-rheumatic treatment strategies on CVD risk could contribute more heavily to the overall CVD risk in the group with shorter disease duration (<10 years disease duration). Subsequently, this skewed distribution could magnify the possible effect of disease duration on CVD risk. However, this would only strengthen our results as we did not find a significant effect of disease duration on CVD risk in spite of this potential source of bias.

Also, as different forms of treatment may be associated with the main independent variable, for example, disease duration and DAS28, or act as a proxy for determinants of the DAS28 such as treatment response and control of disease activity, this relationship is complex. The role of medication on CVD in RA fell outside the scope of this study and was not separately analysed. Investigating the effect of calendar time on CVD would require age-period-cohort analysis using larger cohorts. The effect of these recently adapted treatment strategies on CVD may take some time to become clinically significant. In addition to time trends, missed CVD events due to random misclassification could lead to bias. However, patients enrolled in this cohort are seen and checked regularly (every 3–6 months). Data, including CVD events, are gathered during these check-ups and meticulously recorded in our database. In addition, data of all patients that were included in this study were thoroughly checked and all events that were recorded during follow-up were double-checked to confirm the date of occurrence and type of the event. Deaths were also recorded during follow-up in the database, and for these patients the cause of death was confirmed. Therefore, missed events or misclassification of CVD events was considered an unlikely source of bias in this study.

In conclusion, our results show that disease duration does not appear to independently affect the risk of CVD. Therefore, disease duration may not be the best choice for a disease-specific predictor when estimating CVD risk in individual RA patients, as recently recommended by EULAR. Disease activity over time appears to contribute to the risk of CVD in patients with RA, particularly in case of persistent poorly controlled, high disease activity over time.

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CHAPTER 7

Low disease activity (DAS28≤3.2) reduces the risk of first cardiovascular events in rheumatoid arthritis. A time-dependent Cox regression analysis in a large cohort study

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ABSTRACT

Objective

Systemic inflammation appears to contribute to the excess risk of cardiovascular disease (CVD) in rheumatoid arthritis (RA). The objective of this study was to investigate the effect of different levels of disease activity over time, particularly low disease activity and remission, on CVD risk in patients with RA.

Methods

Data from the Nijmegen early RA inception cohort were used. The primary outcome was first CVD events within the first 10 years of follow-up. Cut points of the DAS28 for remission (<2.6), low (≤3.2), moderate (3.2-5.1), and high (>5.1) disease activity were used. The effect of disease activity on CVD risk was analyzed using Cox-proportional hazards regression with DAS28 as a time-dependent covariate and also conventionally with time-averaged DAS28 as the primary dependent variable.

Results

Low DAS28 (≤3.2) was significantly associated with a reduced risk of CVD (HR 0.65, 95%CI 0.43-0.99) compared to DAS28 >3.2, both when included as a time-dependent covariate and as time-averaged DAS28≤3.2 (HR 0.52, 95%CI 0.33-0.81). Remission had a modest, non-significant protective effect against CVD (HR 0.67, 95%CI 0.43-1.07).

Conclusion

Results of this study suggest that low disease activity is sufficient to achieve a protective effect against CVD in RA. Apparently, remission defined as DAS28<2.6 has no additional protective effect against CVD compared to low disease activity. Our results strengthen the use of tight-control strategies in daily clinical practice to achieve low stable disease activity or remission in RA patients as soon as possible.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease that affects approximately 0.5-1% of the population.[1] RA patients have an increased risk of developing cardiovascular disease (CVD).[2, 3] Evidence suggests the increased cardiovascular risk is partly – in addition to excess of classical risk factors - mediated by systemic inflammation, characteristic for RA. Inflammation may alter the effect of existing CVD risk factors or factors protective for CVD, leading to an increased risk of CVD.[4-6] Furthermore, inflammation may accelerate the atherosclerotic process [7, 8] and lead to the formation of more severe plaques in RA patients.[9-12] In comparison to healthy controls and RA patients in remission, RA patients with active disease seem to have more unstable plaques, which increases the probability of CVD.[13] Consequently, the level of disease activity has been implicated as a contributing factor to the development of CVD, and conversely, the absence of inflammation or clinical remission may be associated with a reduced risk of CVD in RA. The results from a case-control study showed no evidence that disease activity over time was associated with occurrence of myocardial infarction.[14] In a longitudinal study by our group, results indicated that very high disease activity over time or high disease activity at RA onset significantly contributes to the risk of CVD in RA.[15] In a recent study by Myasoedova et al it was demonstrated that particularly exposure to disease activity flare-ups and increased cumulative burden of RA disease activity seems to contribute to this risk.[16] Furthermore, patients with active RA have significantly increased levels of biomarkers for CVD, whilst RA patients who were in remission did not.[17] Overall, these findings led to the hypothesis that achieving a state of remission may reduce the risk of CVD in RA patients. As a clinical consequence, tight control of disease activity could therefore have a beneficial effect on CVD risk.[18] It is unclear whether clinical remission needs to be achieved in order to eliminate or diminish the possible harmful effects of systemic inflammatory activity or if stable low disease activity over time is sufficient. Therefore, the primary objective of this study is to investigate the effect of different levels of disease activity over time, particularly low disease activity and remission, on CVD risk in patients with RA.

PATIENTS AND METHODS

Study design and patients

This is a cohort study using the prospectively collected data from the Nijmegen early RA inception cohort. Patients were included at diagnosis of RA (baseline) in the outpatient clinic of the departments of rheumatology of the Radboud University Medical Centre (since 1985) or the Maartenskliniek in Nijmegen (since 1990). RA patients who fulfilled the 1987 ACR (inclusion before 2010) or ACR/EULAR 2010 (inclusion after 2010) criteria for the classification of RA,[19] with disease duration of <1 year, and who were Disease Modifying Anti-Rheumatic Drug (DMARD) naive were included. All patients received written patient information and gave written informed consent. According to Dutch law and regulations, ethical review was not necessary for this observational study. Patients with confirmed CVD before RA diagnosis and patients with a follow-up <12 months or patients with 2 or less DAS28 measurements were excluded for the current

analyses. All disease activity measurements that were taken between the date of inclusion in the cohort and the date of a first CVD event or censoring were included in the analysis, with a maximum of 10 years of follow-up.

Data collection

The patients were seen during scheduled visits every 3-6 months. During these visits disease activity was measured using the DAS28.[20, 21] Baseline variables were retrieved from the cohort database and included; age (years), gender (male/female), rheumatoid factor (RF) positivity, anti-cyclic citrullinated peptide (anti-CCP) positivity, erythrocyte sedimentation rate (ESR) (mm/hour), C-reactive protein (CRP) (mg/L, Swollen Joint Count (SJC28), Tender Joint Count (TJC28) and the patient Visual Analogue Score [VAS] for global disease activity, DAS28, and Health Assessment Questionnaire (HAQ)). Data on traditional CVD risk factors at baseline were collected by review of medical charts and electronic patient files, including current smoking status (Y/N), blood pressure (mmHg), height (m), weight (kg), diabetes mellitus (Y/N), hypertension (Y/N) and family history of CVD (Y/N). Non-fasting total cholesterol (TC) and high-density-lipoprotein cholesterol (HDL-c) concentrations (mmol/L) were measured using serum from frozen samples collected at baseline at laboratory facilities of Russells Hall Hospital, Dudley UK.[22]

Primary Outcome

The primary outcome was occurrence of a first CVD event (physician diagnosed fatal or non-fatal cases of CVD), as retrieved by extensive review of medical charts and electronic patient files. The following were classified as cardiovascular events: acute coronary syndrome (ACS), stable angina pectoris (AP), cerebral vascular accident (CVA), transient ischemic attack (TIA), peripheral artery disease (PAD) and heart failure (HF). Deaths due to CVD were verified from death certificates, provided by Statistics Netherlands,[23] including deaths due to CVD and CVA but excluding cerebral hemorrhage and non-coronary cardiac death.

Statistical analysis

Baseline variables were compared between the CVD event group and the non-event group by means of independent samples T-test, Wilcoxon or chi-squared statistics. Low disease activity was defined as DAS28 ≤3.2 and clinical remission as DAS28 <2.6.[20, 21, 24]. A Cox-proportional hazard regression model was chosen as the primary analysis with disease activity, low disease activity (DAS28 <3.2) or clinical remission as the segmented time-dependent covariate and time to first CVD event as the primary outcome. This type of analysis is suited to avoid bias introduced by analyzing time course (non-baseline) variables in combination with survival time. Disease activity was measured regularly in patients included in the Nijmegen early RA cohort (every 3-6 months). However as not all patients had measures every 3 months, a six-month interval was maintained for the time-dependent covariate in the Cox-proportional hazard regression analyses. These time segments correspond with DAS28 measurements at six-month intervals. In case of 1 isolated missing measurement, the mean of the measurement prior and the measurement following the missing value was used. If more than 1 consecutive measurement was missing, subjects were censored. To repeat the analysis using a more readily interpretable reflection of

disease activity, the next step was to analyze the time-averaged DAS28 as main dependent variable using conventional Cox-proportional hazard regression analysis. The time-averaged DAS28 was calculated by taking the area under the curve of the DAS28 score of the total follow-up period divided by the follow-up period. The analysis was performed again with the time-averaged DAS28 as a binary variable (time-averaged DAS28 ≤3.2 or 2.6).

In all analyses, sex and age were included in the model as confounders by default. The following potential confounders were considered; current smoking status, baseline measurements of systolic blood pressure (mmHg), diastolic blood pressure (mmHg) and body mass index (BMI) (weight [kg]/height [m]^2), hypertension (physician diagnosis), diabetes mellitus (Type I and II), TC (mmol/L), HDL-c (mmol/L), family history of premature CVD, use of statins and use of anti-hypertensive medication (diuretics, ACE/Angiotensin II inhibitors, beta blockers or calcium blockers) at baseline, RF status, anti-CCP status, baseline DAS28, CRP, and HAQ. Additionally, to further aid the understanding of the relationship between disease activity and CVD risk the effect of both remission and of low DAS28 over time on survival for CVD was assessed using Kaplan-Meier survival analysis. First, subgroups were made based on the time-averaged DAS28; remission (DAS28<2.6), low (DAS28 2.6-3.2), intermediate (DAS28 3.2-5.1) and high (DAS28>5.1). In the second analysis patients were divided using to low time-averaged DAS28 (≤3.2) as the cut-off point. The survival distributions in both analyses were compared using log-rank testing. All analyses were performed using SPSS version 22.0

RESULTS

There were 1157 patients included in the cohort. After exclusion of patients with a prior history of CVD, patients with a follow-up time <12months or patients with 2 or less DAS28 measurements, 873 patients were included in the analyses. A total of 99 RA patients developed a first CVD event during their first 10 years of follow-up. The following fatal and non-fatal first CVD events occurred: 44 (44%) cases of acute coronary syndrome (myocardial infarction or unstable angina pectoris), 18 (18%) cases of stable angina pectoris, 17 (17%) cases of cerebral vascular accident, 5 (5%) patients with a transient ischemic attack, 10 (10%) cases of peripheral artery disease and 5 (5%) patients with heart failure. Out of all CVD events 21% were fatal, mostly due to acute coronary syndrome (43%). Total follow-up time was 4560 patient years with a median (IQR) follow-up time of 5 (3-9) years. At baseline, there were differences between patients with and without CVD events (table 1). Patients with CVD events were on average older, and several other 'traditional' risk factors for CVD were raised including blood pressure, lipids and presence of diabetes. Patients who developed CVD were more frequently RF positive, not more frequently anti-CPP positive and had higher baseline disease activity (table 1). In total 9151 DAS28 measurements were included during follow-up and in 2738 (30%) of the visits, DAS28 was <2.6. Per patient, the percentage of their DAS28 measurements during follow-up that were scored <2.6 (time in remission) was in median (P25-P75) 17% (0.0 %-50%).

Table 1. Patient characteristics at baseline

	Total cohort *N*=873	**No CV event** *N*=774	**CV event** *N*=99	**p-value** (CVD vs. no CVD)
Age (years), mean±SD	54±14	53±14	62±9	**<.001**
Sex (female), n (%)	574(66)	524(68)	50(51)	**0.001**
Currently smoking, n (%)	272(31)	235(30)	69(40)	0.156
BMI (weight[kg]/Height[m]^2), mean±SD	26±4	25±4	26±4	**0.016**
Systolic blood pressure (mmHg), mean±SD	146±24	145±24	153±24	**0.002**
Diastolic blood pressure (mmHg), mean±SD	84±12	83±12	86±11	**0.026**
Hypertension n (%)	120(14)	94(12)	26(26)	**<.001**
Anti-hypertensives, n(%)	134(15)	110(14)	24(24)	**0.009**
Total Cholesterol (mmol/L), mean±SD	5.2±1.2	5.2±1.2	5.3±1.4	0.448
HDL-Cholesterol, mean±SD	1.3±0.3	1.3±0.3	1.2±0.3	**0.040**
TC:HDL-c ratio, mean±SD	4.1±1.0	4.1±1.0	4.4±1.0	**0.013**
LDL-Cholesterol, mean±SD	3.2±1.1	3.1±1.0	3.2±1.2	0.357
Lipid lowering agents, n (%)	30(3.4)	23(3)	7(7)	**0.035**
Diabetes mellitus n (%)	37(4)	29(4)	8(8)	**0.044**
Family history of CVD, n (%)	265(30)	232(30)	33(33)	0.494
Rheumatoid factor (positivity), n (%)	654(75)	576(74)	78(79)	0.345
Anti-CCP (positivity), n(%)	554(64)	493(64)	61(62)	0.686
DAS28, mean±SD	5.0±1.3	4.9±1.3	5.4±1.3	**0.001**
CRP, median (IQR)	14(2-40)	13(2-38)	21(3-47)	0.083
HAQ, median (IQR)	0.6(0.3-1.1)	0.6(0.3-1.1)	0.7(0.3-1.4)	0.468

i. Hypertension is defined as multiple measurements of elevated systolic blood pressure (>140 mmHg) during multiple visits by a physician. Diabetes mellitus includes both type I and type II. All variables represent baseline measures, except when otherwise stated.

ii. BMI; body mass index, HDL; high density lipoprotein, TC; total cholesterol, LDL; low density lipoprotein, CVD; cardiovascular disease, anti-CCP; anti-cyclic citrullinated peptide, DAS28; 28-joint disease activity score, CRP; c-reactive protein, HAQ; health assessment questionnaire.

Cox-proportional hazard regression with a time dependent covariate: disease activity

When disease activity was entered into the model as a continuous, segmented time-dependent variable, the results showed that disease activity had a significant effect on CVD risk after correction for confounders (table 2, panel B), indicating that CVD risk increases as DAS28 increases during follow-up. The hazard ratio (HR), in table 2B of 1.179 can be interpreted as an increase in risk of 18% if the DAS28 is one point higher. Table 2, panel C shows the results from the analysis with DAS28≤3.2 (yes/no) as a time-dependent binary variable after correction for confounders, indicating that CVD risk is significantly lower in patients with DAS28≤3.2 (HR 0.65, 95%CI 0.43-0.99)

Table 2. Cox-proportional hazard regression analysis with time to first CVD event as the primary outcome and time-dependent DAS28 as the primary independent variable, before (panel A) and after (panel B) correction for confounders. Panel C shows results from the Cox-proportional hazards regression with DAS28 <3.2 (yes/no) as a time-dependent covariate after correction for confounders.

	Beta	p-value	HR	95% CI for Exp (B)	
				Lower	Upper
Panel A: Crude model					
Time dependent covariate (DAS28)	**0.113**	**0.119**	**1.120**	**0.972**	**1.290**
Age	0.064	<.001	2.010	1.344	3.005
Gender	0.698	0.001	1.066	1.047	1.085
Panel B: Corrected model					
Time dependent covariate (DAS28)	**0.165**	**0.032**	**1.179**	**1.014**	**1.370**
Age	0.062	<.001	1.064	1.044	1.084
Gender	0.725	0.001	2.065	1.365	3.123
Hypertension baseline	1.036	<.001	2.818	1.673	4.745
HDL-C	-.736	0.043	0.466	0.222	0.977
CV medication†	-.515	0.026	0.597	0.379	0.940
DAS28 baseline	0.034	0.687	1.035	0.877	1.220
Panel C: Corrected model					
Time dependent covariate (DAS28<3.2)	**-.431**	**0.044**	**0.650**	**0.427**	**0.989**
Age	0.064	<.001	1.066	1.046	1.087
Gender	0.736	0.001	2.088	1.372	3.177
Hypertension	0.977	<.001	2.656	1.547	4.559
HDL-C	-1.113	0.009	0.329	0.142	0.758
LDL-c	0.177	0.097	1.193	0.969	1.470
CV medication†	-.541	0.022	0.582	0.366	0.925
CRP	-.001	0.538	0.999	0.994	1.003

i. †Antihypertensive medication, lipid lowering medication.
ii. HDL-c; high-density lipoprotein cholesterol, LDL-c; low-density lipoprotein cholesterol, CRP; c-reactive protein, CV; cardiovascular.

Cox-proportional hazard regression with a time dependent covariate: DAS28 remission

The results from the Cox-proportional hazard analysis with remission (yes/no) as a time-dependent covariate showed a direction for a protective effect of time in remission against CVD (table 3, panel A and B) with a HR of 0.67. However, this effect did not reach statistical significance after correction for confounders (95%CI 0.43-1.07).

Conventional Cox-proportional hazard regression analysis: time-averaged DAS28

Mean±SD time-averaged DAS28 was 3.5±1.1 for the whole group, with a minimum and maximum time-averaged DAS28 of 0.7 and 7.3 respectively. The mean±SD time-averaged DAS28 was significantly lower in the non-event group compared to the event group (3.5±1.1 vs. 3.9±1.2 respectively, with p<0.001). Results from the conventional Cox-proportional hazard regression analysis with time-averaged DAS28 as the main independent variable, showed a significant effect

on CVD risk with a HR of 1.60 (95%CI 1.28-1.99), as shown in table 4, panel B. After correction for confounders, the hazard for CVD notably increases with every point increase in time-averaged DAS28. The results from the following analysis (table 4, panel C) showed that compared to patients with active disease, low time-averaged DAS28 (≤3.2) has a significant protective effect against CVD after correction for confounders (HR 0.53, 95%CI 0.34-0.84). Again, a direction for a protective effect of time in remission against CVD was observed (not shown) with a HR of 0.78. However, this effect did not reach statistical significance after correction for confounders (95%CI 0.45-1.38). These results are in accordance with the results of the first set of analyses that included a time-dependent covariate.

Table 3. Cox-proportional hazard regression analysis with time to first CVD event as the primary outcome and remission (yes/no) as the time-dependent variable, before (panel A) and after (panel B) correction for confounders.

	Beta	p-value	HR	95% CI for Exp (B)	
				Lower	Upper
Panel A: Crude model					
Time dependent covariate (remission)	**-.211**	**0.358**	**0.810**	**0.516**	**1.270**
Age	0.064	0.001	1.066	1.047	1.086
Gender	0.665	0.001	1.945	1.304	2.901
Panel B: correcte model					
Time dependent covariate (remission)	**-.395**	**0.096**	**0.673**	**0.426**	**1.066**
Age	0.063	<.001	1.065	1.045	1.085
Gender	0.680	0.001	1.974	1.306	2.984
Hypertension	0.971	<.001	2.640	1.540	4.528
HDL-C	-.772	0.039	0.462	0.222	0.963
CV medication†	-.526	0.026	0.591	0.372	0.938

i. †Antihypertensive medication, lipid lowering medication.
ii. HDL-c; high-density lipoprotein cholesterol, CV; cardiovascular.

Survival analyses

For illustrative purposes, a Kaplan-Meier survival analysis was performed, investigating the effect of low disease activity and remission on time to first CV event. Patients were divided into four groups; time-averaged DAS28 <2.6, 2.6-3.2, 3.2-5.1, >5.1 for groups 1 through 4 respectively. Event rates were as follows; group 1 (N=189) 16 CV events (8.5%), group 2 (N=163) 14 CV events (8.6%), group 3 (N=444) 55 CV events (12.4%) and group 4 (N=77) 14 CV events (18%). Survival time (time-to-first-CVD event) appears to decrease as time-averaged DAS28 increases (figure 1). Survival distributions differed significantly (p<0.027). Patients with the lowest survival rate (group 4) had the highest baseline DAS28 at diagnosis (mean±SD; 6.1±1.0) compared to group 1, 2 and 3 (mean±SD; 4.1±1.3, 4.8±1.3 and 5.3±1.2 and respectively). Of note, the survival distributions of patients with a time-averaged DAS28 <2.6 and a time averaged DAS28 between 2.6 and 3.2 overlap indicating that these distributions do not differ significantly from each other. Figure 2 shows the survival distributions of patients with a time-averaged DAS28 ≤3.2 or very low disease

activity over time and of patients with more active disease (time-averaged DAS28 >3. 2). Survival distributions (figure 2) differed significantly (p=0.024).

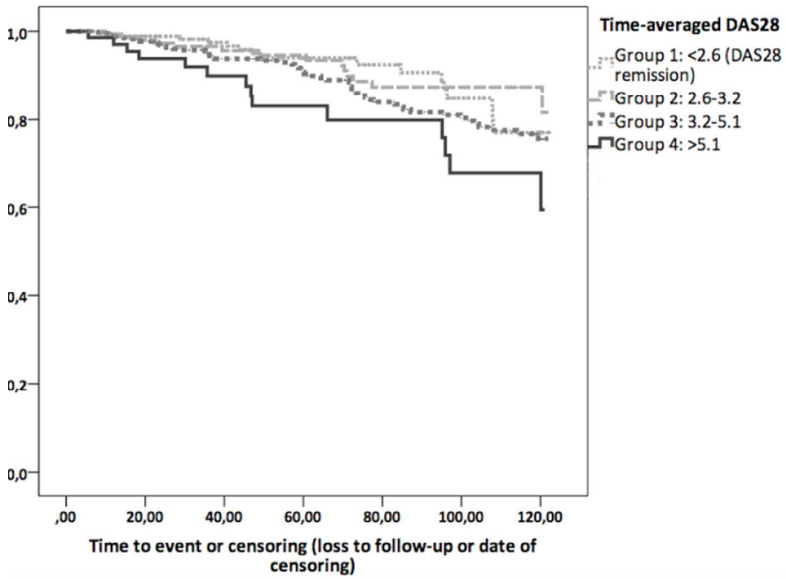

Figure 1. Survival distribution (time to first CVD event) for categories of time-averaged DAS28. Survival distributions differ significantly (p0.027). Cumulative survival of CVD is depicted on the y-axis and time to a CVD event or censoring is depicted on the x-axis

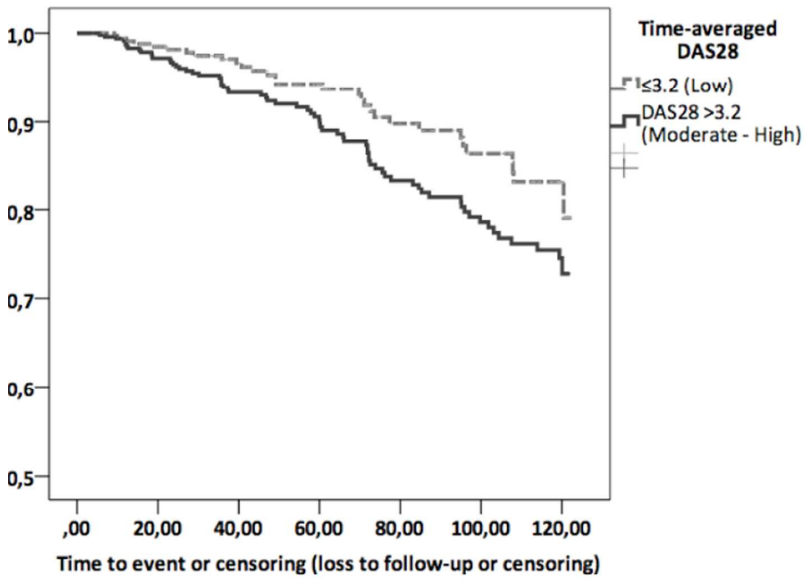

Figure 2. Survival distribution (time to first CVD event) for low (≤3.2) and moderate to high (>3.2) time-averaged DAS28. Survival distributions differ significantly (p<0.001). Cumulative survival of CVD is depicted on the y-axis and time to a CVD event or censoring is depicted on the x-axis.

Table 4. Conventional Cox-proportional hazard regression analysis with time to first CVD event as the primary outcome and time-averaged DAS28 as the primary independent variable, before (panel A) and after (panel B) correction for confounders and included as a binary variable (panel C).

	Beta	p-value	HR	95% CI for Exp (B)	
				Lower	Upper
Panel A: Crude model					
Time-averaged DAS28	**0.383**	**<0.001**	**1.466**	**1.204**	**1.786**
Age	0.060	<0.001	1.062	1.043	1.082
Gender	0.848	<0.001	2.336	1.549	3.521
Panel B: full model					
Time-averaged DAS28	**0.468**	**<0.000**	**1.597**	**1.279**	**1.994**
Age	0.056	<0.001	1.057	1.037	1.077
Gender	0.954	<0.001	2.595	1.712	3.933
Hypertension baseline	0.920	<0.001	2.508	1.566	4.018
DAS28 baseline	-.048	0.587	0.953	0.802	1.133
Panel D: full model					
Time-averaged DAS28 binary; (≤3.2)	**-.630**	**0.007**	**0.533**	**0.337**	**0.843**
Age	0.058	<0.001	1.060	1.040	1.080
Gender	0.803	<0.001	2.231	1.491	3.339
Hypertension baseline	0.882	<0.001	2.417	1.506	3.879
DAS28 baseline	0.042	0.614	1.043	0.886	1.228

i. DAS28; 28-joint disease activity score

DISCUSSION

Systemic inflammatory activity in RA has been suggested as an important contributing factor to the excess CVD risk in RA patients. Therefore, it was hypothesized that achieving a state in which disease activity is low or nearly absent could have a beneficial effect on CVD risk. In this study, it is shown that low stable disease activity over time has a significant protective effect against CVD in RA. Although clinical remission (DAS28<2.6) appears to have a modest protective effect against developing CVD, it did not reach statistical significance.

Previous studies have demonstrated that inflammation contributes to accelerated atherosclerosis.[25-27] Atherosclerosis in turn is an intermediate in causing non-bleeding CV events. Concordantly, the results of this study show that active disease in RA is associated with an increased risk of developing CV events. Furthermore, when looking at the overall trend of disease activity during follow-up, those patients who were able to achieve and maintain low disease activity over time appear to have a significantly lower risk of CVD than patients with more active disease. Interestingly, it appears that achieving remission does not offer any significant added value over sustained low disease activity, in terms of CVD risk reduction. Time to first CVD event was similar in patients with low disease activity and patients in remission and these patients

had a significantly longer survival time compared to patients with more active disease. The results also showed that patients with the lowest survival times for CVD had the highest disease activity levels at baseline suggesting very active disease onset in these patients. Several other studies have reported similar results with regards to increased disease activity in RA.[15, 16, 28] In a large RA cohort study, CRP was found to be associated with the risk of CVD although DAS28 did not appear to be significantly increased in RA patients with MI compared to RA controls.[29] However, only the first 6 months of follow-up after inclusion were incorporated for disease activity in this study. By contrast, Myasoedova et al. have shown in a recent study that particularly bouts of uncontrolled high disease activity are associated with a higher risk of CVD.[16] A study by Solomon et al. also included longitudinal disease activity, demonstrating that reduced time-averaged disease activity in RA is associated with fewer CVD events.[28] What our current study adds to that is notably longer follow-up with detailed data on determinants of CVD risk. Also, in this study a Cox-proportional hazards regression with time-dependent covariates was used, as conventional Cox regression analysis using DAS28 values after baseline is potentially biased.[30] With regards to remission, another study demonstrated that RA patients in remission, defined as Clinical Disease Activity Index, or CDAI ≤2.8 had significantly lower levels of CVD risk markers compared to patients with active disease, supporting remission as a target for CVD risk management in RA.[17] Overall, patients who are able to achieve and maintain remission or low disease activity during follow-up, even sporadically, may be less likely to develop bouts of uncontrolled, sustained high systemic inflammation, a contributing factor to atherosclerosis and CVD. RA patients with very active disease at diagnosis, poor treatment response with more frequent flare-ups as a result may form a subgroup within the RA population that is particularly at risk for developing CVD, significantly contributing to the excess CVD risk in this population.

In the analysis of the effect of low disease activity or clinical remission on CVD risk, there are several statistical considerations. Patients that were able to achieve frequent, longstanding DAS28 remission comprised a relatively small group in this study which could have contributed to the fact that a protective trend against CVD was observed but did not reach statistical significance. Disease activity tends to fluctuate over the course for RA, which makes it difficult to accurately capture the level of exposure of a patient during follow-up. Also, as noted above, there may be a risk of bias as the patients who are able to stay event free the longest, also have inherently more time to achieve remission or low disease activity, creating a survivor bias or 'immortal time bias'. Immortal time bias can be avoided by integrating the changes in exposure status in the analysis.[30] Consequently, for this study a Cox-proportional hazards regression with a segmented time dependent covariate (DAS28) was chosen. Furthermore, in RA, different measures of disease activity provide various definitions of remission,[20, 31-34] which were not all considered in this study. These definitions do appear to strongly correlate with each other,[32, 33] however including a different definition for remission could have an effect on results. DAS28 remission that was used in this study is defined as disease activity score <2.6 and this is not the same as the absolute absence of disease activity. On the other hand, remission according to the stricter ACR/EULAR remission criteria for RA is not prevalent, yet. Additional research is needed

to determine if the complete absence of disease activity has a significant added protective effect on CVD risk compared to very low disease activity.

In conclusion, low disease activity appears to have a significant protective effect against CVD in RA. Achieving sustained remission, here defined as DAS28<2.6, is regarded as the ultimate treatment target but does not seem to provide a large advantage over low disease activity over time in terms further reducing CVD risk. RA patients with uncontrolled high disease activity appear to have the highest risk of developing CVD. Our results strengthen the use of tight-control (treat-to-target) strategies in daily clinical practice to achieve low disease activity or remission in these patients, also with the aim to reduce CVD risk.

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CHAPTER 8

Prediction of cardiovascular risk in rheumatoid arthritis: performance of original and adapted SCORE algorithms

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Annals of Rheumatic Diseases
2016; 75: p 674-680

ABSTRACT

Objective

Predictive performance of cardiovascular disease (CVD) risk calculators appears suboptimal in rheumatoid arthritis (RA). A disease-specific CVD risk algorithm may improve CVD risk prediction in RA. The objectives of this study are to adapt the Systematic COronary Risk Evaluation (SCORE) algorithm with determinants of CVD risk in RA and to assess the accuracy of CVD risk prediction calculated with the adapted SCORE algorithm.

Methods

Data from the Nijmegen early RA inception cohort were used. The primary outcome was first CVD events. The SCORE algorithm was recalibrated by re-weighing included traditional CVD risk factors and adapted by adding other potential predictors of CVD. Predictive performance of the recalibrated and adapted SCORE algorithms was assessed and the adapted SCORE was externally validated.

Results

Of the 1016 included patients with RA, 103 patients experienced a CVD event. Discriminatory ability was comparable across the original, recalibrated and adapted SCORE algorithms. The Hosmer–Lemeshow test results indicated that all three algorithms provided poor model fit ($p<0.05$) for the Nijmegen and external validation cohort. The adapted SCORE algorithm mainly improves CVD risk estimation in non-event cases and does not show a clear advantage in reclassifying patients with RA who develop CVD (event cases) into more appropriate risk groups.

Conclusions

This study demonstrates for the first time that adaptations of the SCORE algorithm do not provide sufficient improvement in risk prediction of future CVD in RA to serve as an appropriate alternative to the original SCORE. Risk assessment using the original SCORE algorithm may underestimate CVD risk in patients with RA.

INTRODUCTION

In rheumatoid arthritis (RA), cardiovascular disease (CVD) morbidity and mortality are increased.[1, 2] Inflammation may contribute to the increased risk of CVD,[3–10] suggesting that inflammatory markers should be incorporated in CVD risk prediction models for RA. For example, high-sensitivity C reactive protein (CRP) was included in the recently developed Reynolds Risk Score (RRS), which is therefore better able to classify premenopausal women into different risk groups.[11, 12] Similarly, disease activity measures, such as the 28-joints Disease Activity Score (DAS28), may be useful in CVD risk algorithms for patients with RA. Additionally, inflammation may add and modulate traditional CVD risk factors.[13, 14] Existing weights attributed to each individual risk factor included in currently available CVD risk algorithms may therefore require adjustment. The use of other CVD-related parameters not incorporated in the present CVD risk algorithms, such as carotid artery intima-media thickness and/or the presence of atherosclerotic carotid plaques in these patients, may also be considered.[15–17] Current CVD risk prediction models, for example, Framingham Risk Score (FRS) or the Systematic COronary Risk Evaluation (SCORE) algorithm, have been developed for use in the general population.[18–20] Their performance in patients with RA appears to be suboptimal.[21–23] Therefore, it has been suggested that CVD risk algorithms based solely on traditional risk factors may not be suited for use in the RA population. As a first step towards more accurate CVD risk prediction, it was proposed in the European League Against Rheumatism (EULAR) recommendations for CVD risk management in RA to apply a multi- plication factor of 1.5 to the calculated CVD risk by SCORE in selected patients to enhance the risk estimates.[24] However, recent studies have shown that this multiplication factor does not significantly improve CVD risk prediction in patients with RA.[25–27] A disease-specific CVD risk algorithm could perhaps improve prediction of CVD risk in patients with RA. In Europe, the SCORE algorithm is widely used. A logical next step would be to evaluate whether the SCORE algorithm can be adapted to more accurately estimate the risk of CVD in patients with RA. Therefore, the objectives of this study were (1) to adapt the SCORE algorithm with determinants of CVD risk in patients with RA and (2) to compare the performance of the modified SCORE calculator to the original SCORE risk algorithm with regards to CVD risk prediction in patients with RA.

PATIENTS AND METHODS

Study design and patients

Data from the Nijmegen early RA inception cohort were used for this study. Patients were included at diagnosis of RA (baseline) in the outpatient clinic of the Departments of Rheumatology of the Radboud University Medical Centre (since 1985) or the Maartenskliniek (since 1990) in Nijmegen, The Netherlands. Patients with RA who fulfilled the 1987 American College of Rheumatology (ACR; inclusion before 2010) or ACR/EULAR 2010 criteria (inclusion after 2010) for the classification of RA,[28] with disease duration of <1 year, and who were disease-modifying antirheumatic drug naive were included. All patients gave written informed consent. Patients with a history of confirmed CVD before RA diagnosis and patients with a follow-up <18 months were

excluded. Patients were censored after 10 years of follow-up or at the time of the first CVD event. The 10-year risk estimates for patients with a follow-up <10 years at the time of censoring (30 September 2011) were adjusted proportionally according to the actual follow-up time and calculated as a proportion of 10 years.[22] The SCORE algorithm for the prediction of both fatal and non-fatal disease in the Dutch population was used.[20]

Baseline predictors of cardiovascular disease

In addition to the traditional risk factors used in the SCORE algorithm, potential RA-specific predictors for CVD were collected. Baseline characteristics were retrieved from the cohort database including; age (years), gender (male/female), rheumatoid factor (RF) status, anti-cyclic citrullinated peptide (anti-CCP) status, DAS28, erythrocyte sedimentation rate (ESR; mm/hour), swollen joint count (SJC), tender joint count (TJC) and the patient Visual Analogue Score (VAS) for global disease activity, Health Assessment Questionnaire (HAQ) and CRP (mg/L). Data on traditional CVD risk factors at baseline were collected by medical chart and electronic patient file review, including current smoking status (Y/N), blood pressure (mm Hg), height (m), weight (kg), diabetes mellitus (Y/N), hypertension (Y/N) and family history of premature CVD (Y/N). Lipid levels were measured using serum from frozen samples collected at baseline. Non-fasting total cholesterol (TC) and high-density-lipoprotein cholesterol (HDL-c) concentrations (mmol/L) were measured using laboratory facilities of Russells Hall Hospital, Dudley, UK.

Primary outcome

The primary outcome was first CVD events (physician diagnosed fatal or non-fatal CVD events), which were retrieved by extensive review of medical charts and electronic patient files. We included the following CVD events: acute coronary syndrome (myocardial infarction and unstable angina pectoris), cerebral vascular accident (CVA) and heart failure (HF). Deaths due to CVD were verified from death certificates, provided by Statistics Netherlands,[29] including deaths due to CVD and CVA but excluding cerebral hemorrhage and non-coronary cardiac death.

Statistical analysis

The analysis consisted of two phases: (1) the recalibration and adaptation of the SCORE algorithm in our cohort and (2) evaluation of the predictive performance of the recalibrated and adapted SCORE algorithms. For the recalibration of the SCORE algorithm, the regression coefficients (the weights) of the predictors originally included in SCORE (current smoking status, systolic blood pressure and TC:HDL-c ratio) were newly estimated by means of Cox-proportional hazards regression analysis. The SCORE algorithm as developed by Conroy et al.[18] is fit for use in patients aged ≤65 years for the prediction of fatal CVD. Van Dis et al.[20] recalibrated the SCORE for the prediction of fatal and non-fatal CVD in the Dutch population and for individuals both aged ≤65 and >65 years. The SCORE algorithm was incorporated in the most recent version of the Dutch national guideline for cardiovascular risk management and we used this version.[30] For the adaptation of the SCORE algorithm, other potential predictors were added to the existing SCORE variables. Variables with a significance level $p<0.1$ in univariate Cox proportional hazards regression analysis were evaluated in a multivariate Cox proportional hazard regression analysis.

In the multivariate analysis, traditional CVD risk factors included in the SCORE algorithm were predetermined to stay in the model and other new predictors were included at a deliberately more liberal p value of <0.2. The following potential risk factors were considered: body mass index (BMI; weight (kg)/ height (m)2), hypertension (physician diagnosis), diabetes mellitus (type I and II), diastolic blood pressure (mmHg), TC (mmol/L), HDL-c (mmol/L), low-density lipoprotein cholesterol (mmol/L), triglycerides (mmol/L), family history of premature CVD, RF status, anti-CCP status, DAS28, CRP, ESR (mm/hour), SJC, TJC, VAS and HAQ. All analyses were performed with CVD events as the dependent variable and follow-up time (time since RA diagnosis) as the time variable. Discrimination, that is, the number of patients who are correctly grouped into the event and the non-event group, was tested using the concordance statistic (c-statistic) and the area under the receiver operating characteristic (ROC) curve.[31] Calibration was assessed by comparing the agreement between the observed number of CVD events and the number of CVD events predicted by the SCORE algorithms, in deciles of predicted CVD risk. Hosmer–Lemeshow (H–L) tests and calibration plots were used to assess model fit. Clinical relevance of the models was assessed based on the number of patients reclassified into another CVD risk group: <10% (low risk), 10%–20% (intermediate risk) and >20% (high risk).[20, 30] The analyses were performed using SPSS V.21.0. Missing values on individual variables were imputed using multiple imputations with five repetitions.

External validation

The performance of the original, recalibrated and adapted SCORE algorithms concerning the prediction of fatal and non-fatal CVD was also analyzed in external cohorts, consisting of 400 patients with RA from the UK (DRACCO cohort) and 204 patients from a Norwegian cohort (EURIDISS/ORAreg).[32, 33]

RESULTS

A total of 141 patients with a follow-up time of <18 months or documented CVD prior to RA diagnosis were excluded, leaving 1016 patients for analysis (table 1). During follow-up, 103 first CVD events occurred, including cases of acute coronary syndrome (n=66), ischaemic stroke (n=26), HF (n=4) and CVD deaths (n=7). At the time of event or censoring, patients had a mean±SD disease duration of 7.8±3.5 years.

Model development

When recalibrating the SCORE algorithm only the traditional CVD risk factors were included in the Cox-proportional hazard regression, changing the regression coefficients (weights) as a result. For the adaptation of the SCORE model new variables were added (please see the supplementary tables S1 and S2 for further information). The following CVD risk factors were significant predictors: current smoking status, systolic blood pressure, TC:HDL ratio, BMI, diabetes mellitus at baseline, hypertension at baseline, high baseline DAS28 (cut-off point >5.1). The median (IQR) 10-year CVD risk scores calculated by the original and the adapted SCORE algorithm were 9.1% (2.7%–26.6%) and 6.7% (1.6%–18.4%) respectively.

Table 1. Patients characteristics

	Total cohort (n = 1016)	No CVD (n= 913)	CVD (n = 103)	p-value
Age (years), mean±SD	54±13	53±14	62±10	**<.001**
Sex (female), n(%)	674 (66.3)	620 (67.9)	54 (52.4)	**0.002**
Currently smoking, n(%)	313 (30.8)	280 (30.7)	40 (38.8)	0.133
BMI (weight(kg)/height(m)^2), mean±SD	25.5±4.3	25.5±4.3	26.9±4.1	**0.004**
Systolic blood pressure (mm Hg), mean±SD	146±24	145±24	156±25	**<.001**
Diastolic blood pressure (mm Hg), mean±SD	84±11	84±12	87±9	**0.004**
Hypertension (physician diagnosis)				
At baseline, n(%)	144 (14.2)	117 (12.8)	27 (26.2)	**<.001**
During follow-up, n(%)	260 (25.6)	220 (24.1)	40 (38.8)	**0.002**
Antihypertensives, n(%)	159 (15.6)	130 (14.2)	29 (28.2)	**<.001**
Total cholesterol (mmol/L), mean±SD	5.2±1.3	5.2±1.3	5.2±1.3	0.743
HDL-cholesterol, mean±SD	1.3±0.3	1.3±0.3	1.2±0.3	**0.018**
TC:HDL-c ratio, mean±SD	4.2±1.0	4.1±1.1	4.5±1.2	**0.037**
LDL-cholesterol, mean±SD	3.2±1.1	3.2±1.0	3.2±1.1	0.526
Non-HDL cholesterol, mean±SD	3.9±1.1	3.9±1.1	4.0±1.2	0.296
Lipid-lowering agents, n(%)	40 (3.9)	33 (3.6)	7 (6.8)	0.191
Diabetes mellitus				
At baseline, n(%)	44 (4.3)	36 (3.9)	8 (7.8)	0.121
During follow-up, n(%)	84 (8.3)	69 (7.6)	15 (14.6)	**0.024**
Family history of CVD, n(%)	312 (30.7)	274 (30.0)	38 (36.9)	0.180
Rheumatoid factor (positivity), n(%)	761 (74.9)	680 (74.5)	81 (78.6)	0.427
Anti-CCP (positivity), n(%)	643 (63.3)	592 (64.8)	68 (66)	0.803
DAS28, mean±SD	4.9±1.3	4.9±1.3	5.3±1.3	**0.002**
Swollen joint count, median (IQR)	8 (5–12)	8 (5–12)	9 (5–13)	0.104
Tender joint count, median (IQR)	5 (3–10)	5 (3–10)	7 (2–12)	0.057
ESR, median (IQR)	25 (16–45)	24 (16–45)	35 (21–50)	**0.002**
VAS, median (IQR)	41 (30–57)	40 (29–56)	48 (32–65)	**0.008**
CRP, median (IQR)	16 (3–42)	15 (3–41)	26 (6–48)	**0.023**
HAQ, median (IQR)	0.6 (0.3–1.1)	0.6 (0.3–1.1)	0.8 (0.3–1.1)	0.054
MTX treatment ever, n(%)	555 (54.6)	506 (55.4)	49 (47.6)	0.158
Biological DMARDs ever, n(%)	221 (21.8)	213 (23.3)	8 (7.8)	**<.001**

i. Hypertension is defined as multiple measurements of elevated systolic blood pressure (>140 mm Hg) during multiple visits by a physician. Diabetes mellitus includes both type I and type II.

ii. All variables represent baseline measures, except when otherwise stated.

iii. Anti-CCP, anti-cyclic citrullinated peptide; BMI, body mass index; CRP, C reactive protein; CVD, cardiovascular disease; DAS28, 28-joint disease activity score; DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MTX, methotrexate; TC, total cholesterol; VAS, Visual Analogue Scale (patient global VAS).

Discrimination

Discriminatory performance was comparable across the original, recalibrated and adapted SCORE algorithms, with an area under the curve of 0.78 (95% CI 0.74 to 0.82), 0.78 (0.74 to 0.83) and

0.80 (0.75 to 0.84), respectively. The corresponding ROC curves for the original and adapted SCORE are presented in figure 1. The c-statistic values were similar between 0.75 and 0.76 for all three algorithms.

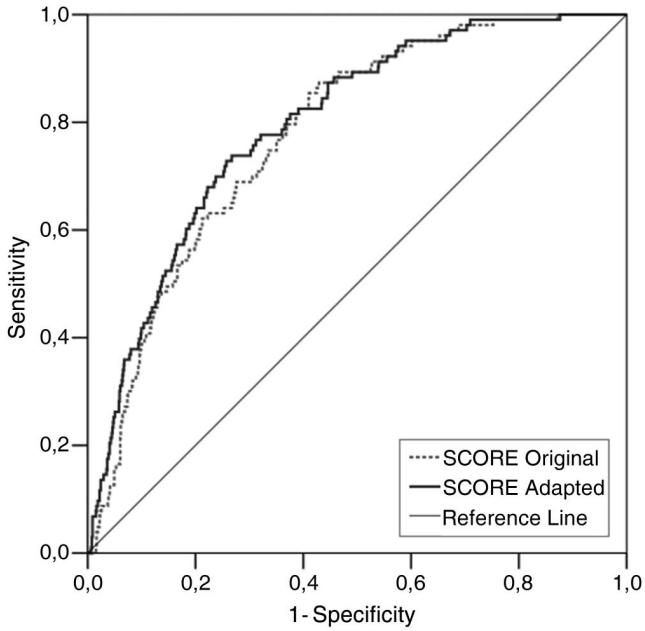

Figure 1. Receiver operating characteristic (ROC) curves for the original Systematic COronary Risk Evaluation (SCORE) and the adapted SCORE algorithms. Area under the curve values were (95% CI) 0.78 (0.74 to 0.82) and 0.80 (0.76 to 0.84) for the original and adapted SCORE algorithm, respectively.

Calibration

Patients were grouped into deciles based on ascending predicted CVD risk. In each of these groups the observed number of CVD events was compared with the calculated (expected) risk for CVD events. It appeared that when using the original SCORE algorithm, the CVD risk was underestimated in the lower and middle deciles and was greatly overestimated in the top decile (figures 2A and 3A). The H–L test indicated a poor model fit with a p value of <0.001. Next, the recalibrated algorithm that included only the 're-weighted' traditional risk factors underestimated CVD risk across all deciles with a p value of <0.001 for the H–L test, also indicating poor model fit (not shown). Then, the adapted SCORE algorithm underestimated CVD risk in the lower and middle deciles and overestimated CVD risk in the highest decile, with a p value for the H–L test of 0.04 (figures 2B and 3B).

Reclassification of CVD risk

For clinical care, patients usually are classified into risk groups. The number of patients classified by the original SCORE and the adapted SCORE in each of the three risk groups (>10%, 10%–20% and >20% CV risk) is presented in tables 2 and 3. Although the clinically important 10%–20% risk

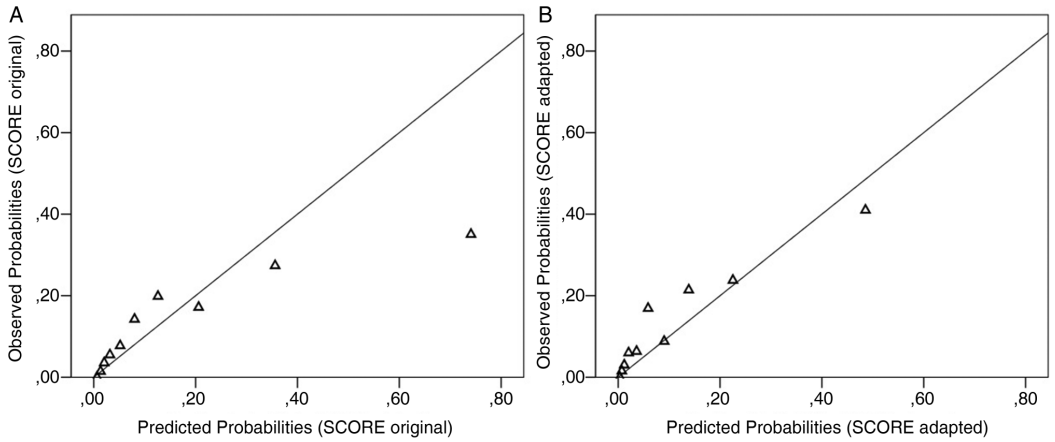

Figure 2. Calibration plots. Observed probabilities depicted at the y axis, and the predicted probabilities depicted at the x axis as calculated by the original Systematic COronary Risk Evaluation (SCORE) algorithm (A) and the adapted SCORE algorithm (B). A line was fitted between the observed and predicted probabilities of cardiovascular disease events using cubic spline.

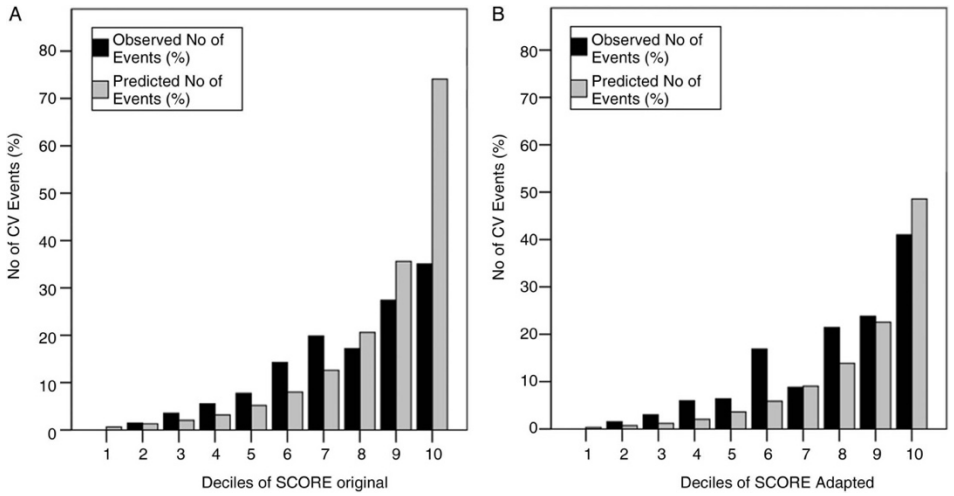

Figure 3. Bar chart with the observed number of events (%) depicted at the y axis, and the deciles of predicted risk depicted at the x axis as calculated by the original Systematic COronary Risk Evaluation (SCORE) algorithm (A) and the adapted SCORE algorithm (B). CV, cardiovascular.

group did increase in numbers for the adapted SCORE, not all patients with a CVD event were reclassified appropriately because their predicted risk remained too low. In total, six patients with an event were reclassified to the low-risk group by the adapted SCORE compared with seven patients who were correctly reclassified to a higher risk group. The adapted SCORE model did perform noticeably better reclassifying the non-event patients (i.e., their predicted risk became appropriately lower). Overall, estimates of CVD risk by the original SCORE and the adapted SCORE algorithms were similar for the majority of patients and did not lead to a reclassification into another risk group for most patients (68%).

Table 2. Patients grouped per CVD risk category for the original SCORE and the adapted SCORE

	Original SCORE			Adapted SCORE		
n(%)	<10%	10-20%	>20%	<10%	10-20%	>20%
CVD						
(n = 103)	**24 (23.3)**	**17 (16.5)**	**62 (60.2)**	**27 (26.2)**	**24 (23.3)**	**52 (50.5)**
No CVD						
(n = 913	**586 (64.2)**	**137 (15.0)**	**190 (20.8)**	**658 (72.1)**	**130 (14.2)**	**125 (13.7)**

i. CVD, cardiovascular disease; SCORE, Systematic COronary Risk Evaluation.

Table 3. CVD risk group reclassification of RA patients when using the original SCORE and the adapted SCORE algorithms

CVD (n = 103)	Adapted SCORE			
Original SCORE		<10%	10-20%	>20%
	<10%	21 (20.4)	2 (1.9)	1 (1.0)
	10%–20%	4 (3.9)	9 (8.7)	4 (3.9)
	>20%	2 (1.9)	13 (12.6)	47 (45.6)
No CVD (n = 913)	**Adapted SCORE**			
Original SCORE		<10%	10-20%	>20%
	<10%	570 (62.4)	14 (1.5)	2 (0.2)
	10%–20%	66 (7.2)	57 (6.2)	14 (1.5)
	>20%	22 (2.4)	59 (6.5)	109 1.9)

i. CVD, cardiovascular disease; SCORE, Systematic COronary Risk Evaluation.

External validation

After exclusion of 93 patients with a history of CVD at baseline, 511 patients were included for external validation (please see supplementary table S3 for further details). Patients had a mean±SD follow-up of 7.5±2.2 years. A total of 26 first fatal or non-fatal CVD events were registered including 17 acute coronary events, five cases of stroke, two cases of HF and two cases of other CV death. Due to missing values on non-traditional CVD risk factors, 24 events were available for analysis. Missing values ranged from 0% to 5% (DAS28). Discriminatory ability of the adapted SCORE algorithm was inferior to the original SCORE algorithm with an area under the curve of 0.76 (95% CI 0.68 to 0.84), 0.74 (0.66 to 0.83) for the original and adapted SCORE algorithm, respectively. The H–L test indicated a poor model fit for both models with a p value of <0.001.

DISCUSSION

In RA, risk assessment by traditional CVD risk models such as SCORE appears to be suboptimal.[21 22 27] Therefore, the aim of this study was to adapt the SCORE algorithm to improve the accuracy of CVD risk estimates in patients with RA, by changing the weights (recalibration) and by adding new variables (adaptation). Unfortunately, recalibration and adaptation of the SCORE algorithm with additional RA-specific CVD risk factors did not lead to major improvements in the accuracy of CVD risk prediction in patients with RA.

Several RA-specific predictors were considered and some of them, such as high DAS28 at baseline, showed significant predictive power. However, in the end the SCORE algorithm adapted with RA-specific predictors showed a rather modest improvement in discriminatory ability in comparison to the original SCORE. Furthermore, the adapted SCORE algorithm mainly improved overestimation of CVD risk in patients without CVD and in the highest CVD risk groups. Overestimation of CVD risk may be harmful as patients receive unnecessary treatment. However, mostly intermediate-risk to high-risk patients were affected by CVD risk overestimation, in which case this would only reaffirm treatment indication and would not change the indication overall. Improvement of CVD risk estimates in these patients is therefore less important for clinical purposes. Much could be gained from improving the classification of patients with RA who later develop CVD (event cases) into higher risk groups so these patients become eligible for preventive treatment. The adapted SCORE does not show a significant improvement in this area, leaving undetected high-risk patients with RA at risk for being under treated. In general, underestimation of CVD risk in RA appears to be the main problem with the original SCORE as shown by us as well as by others.[21 27] Similar results have been reported for other CVD risk calculators such as FRS.[22] The FRS significantly underestimated CVD risk, especially in older patients and in patients with positive RF and persistently elevated ESR. This indicates that disease severity and inflammation, which are not accounted for in current CVD risk algorithms, may play a role in CVD risk prediction in patients with RA. However, our adaptation of the SCORE algorithm including these variables did not solve the issue. It is not clear why CVD risk generally is underestimated in RA. One explanation may be that the atherosclerotic burden in patients with RA is not always mirrored by the SCORE or other risk calculators.[23, 25, 34] In patients with RA, the SCORE risk estimates did not associate well with subclinical carotid atherosclerosis[25] and patients with RA with high coronary artery calcification were infrequently assigned to be at elevated risk by the FRS or the RRS.[23] Calcifications and plaques of coronary arteries also occurred in patients with RA classified as having low CV risk (<1%) according to the SCORE.[34] Therefore, in RA, important subclinical atherosclerosis is not reflected in CVD risk when applying these risk calculators.

This study has several limitations. For one, the external validation cohort consists of patients with both early and established RA from varying geographical areas. Conflicting results have been reported with regards to the onset of the increased risk of CVD in patients with RA.[24, 35–38] It may prove to be difficult to develop a singular CVD risk algorithm that can be applied successfully in all RA populations across different countries. Furthermore, the baseline risk that was determined in the general population for the original SCORE was also included in the adapted algorithm and this may contribute to systematic underestimation of CVD risk by this algorithm. As the baseline risk for CVD is increased in an individual with RA compared to a healthy counterpart of similar age and sex,[1, 2] it may be necessary to adapt this baseline risk. However, although our cohorts are among the largest RA cohorts available with sufficient data on CVD risk factors and follow-up, the number of available patients with RA and CVD events in this individual cohort was still deemed insufficient to determine a reliable, robust baseline risk that could be extrapolated to other RA populations. Furthermore, the adapted SCORE algorithm developed in this study is a basic revision of the original SCORE including all traditional CVD risk

factors already included in the original SCORE. Although this approach is suitable for smaller cohorts it also comes with restrictions in terms of predictor selection. The structure and format of the SCORE are largely maintained, regardless of whether this would provide the best fit for the RA population. Hypothetically, some of the well-established traditional risk factors that form the base of the CVD risk algorithms that are currently used, may require replacement by other, stronger, RA-specific predictors of CVD risk. These predictors may be more sensitive to the subtle differences between low-risk and high-risk patients in the RA population. The results of this study showed a significant relationship between high baseline disease activity and CVD risk. This is in concurrence with other research.[17, 38]

In conclusion, this study demonstrates that adaptations of the SCORE algorithm did not provide sufficient improvement in the predictive performance to serve as an appropriate alternative to the original SCORE for the prediction of the 10-year risk of CVD in RA. A larger cohort with a higher number of CVD events might be used to develop a RA-specific CVD risk algorithm taking into consideration other factors, most intuitively related to the disease pathogenesis and inflammation. Alternatively, additional investigations such as carotid ultrasound may provide a substantial improvement of correct classification of these patients, even when using the original SCORE. Future studies should address these hypotheses to shed more light onto this matter and contribute to more efficient CVD risk management in RA, eventually decreasing CVD morbidity and mortality in this population.

Supplementary tables

Table S1. Results from the multivariate Cox-proportional hazard regression analysis.

	B	P-value	OR	95% CI for OR	
				Lower	Upper
SCORE Recalibrated ≤65 years					
Smoking	0.341	0.165	1.406	0.865	2.285
Systolic blood pressure *	0.019	<0.001	1.019	1.011	1.027
TC:HDL-c ratio*	1.407	0.004	4.085	1.574	10.60
SCORE Recalibrated >65 years					
Age*	0.061	<0.001	1.063	1.044	1.082
Sex	0.570	0.005	1.769	1.188	2.634
Smoking	0.382	0.148	1.465	0.867	2.473
Systolic blood pressure*	0.008	0.061	1.008	1.000	1.017
TC:HDL-ratio	1.228	0.011	3.414	1.324	8.806
SCORE Adapted					
Smoking	0.380	0.115	1.462	0.909	2.350
Systolic blood pressure*	0.012	0.009	1012	1.003	1.021
TC:HDL-c ratio*	1.387	0.006	4.005	1.488	10.78
BMI	0.038	0.068	1.039	0.997	1.082
Diabetes at baseline	0.695	0.068	2.004	0.949	4.233
Hypertension at baseline	0.725	0.003	2.065	1.273	3.348
DAS28 >5.1	0.557	0.006	1.745	1.170	2.601

i. TC; total cholesterol, HDL-c; high-density lipoprotein cholesterol, BMI; body mass index, DAS28; 28-joint disease activity score, OR; odds ratio, CI; confidence interval

Table S2. SCORE recalibrated and adapted SCORE algorithms

Recalibrated SCORE:

*≤65 years old:

SCORE recalibrated = 1-Baseline Risk ** EXP[0.019 x (systolic blood pressure - 140 mmHg) + 1.407 x natural logarithm (TC:HDL-c - 1.061)+0.340824 (if currently smoking)] x 100%

*>65 years old:

SCORE recalibrated = 1 - 0.93884 ** EXP[0.570 (if male) + 0.061 * (age - 55) + 0.382 (if currently smoking) + 0.008 x (systolic blood pressure - 137 mmHg) + 1.228 x natural logarithm (TC:HDL- 1.608)] x 100%.

Adapted SCORE:

SCORE updated = 1-Baseline Risk ** EXP(0.012 x (systolic blood pressure -140 mmHg) + 1.387 x natural logarithm (TC:HDL-c - 1.061) + 0.379527 (if currently smoking) + 0.038 x BMI + 0.695 (in case of diabetes mellitus) + 0.725 x (in case of hypertension) + 0.557 (if DAS28 > 5.1))*100.

i. SCORE; Systemic COronary Risk Evaluation, TC; total cholesterol, HDL-c; high-density lipoprotein cholesterol, BMI; body mass index, DAS28; 28-joint disease activity score, OR; odds ratio, CI; confidence interval

Table S3. Patient characteristics of the external validation cohort.

	Whole group (n=511)	CV event (n=26)	No CV event (N=485)
Age (years), mean±SD	60±12.3	69±9	60±12
Sex (female), n(%)	393 (77)	16 (62)	377 (78)
Smoking, n(%)	111 (22)	4 (15)	107 (22)
BMI (weight[kg]/Height[m]^2), mean±SD	26.9±5.1	26±3.5	26.9±5.1
Systolic blood pressure (mmHg), mean±SD	144±23	155±24	143±22
Diastolic blood pressure (mmHg), mean ±SD	81±12	81±12	81±12
Hypertension, n(%)	340 (66.5)	23 (89)	317 (65)
Treated with anti-hypertensives, n(%)	162 (32)	13 (50)	149 (31)
Total Cholesterol (mmol/L), mean±SD	5.5±1.2	6.1±1.2	5.4±1.2
HDL-Cholesterol, mean±SD	1.7±0.5	1.7±0.5	1.7±0.5
TC:HDL-c ratio, mean±SD	3.6±1.1	3.8±1.1	3.5±1.1
LDL-Cholesterol, mean±SD	3.2±1.2	3.8±1.2	3.2±1.1
Treatment with statins/fibrates, n(%)	60 (12)	3 (12)	57 (12)
Diabetes, n(%)	36 (7)	1 (4)	35 (7)
Rheumatoid factor (positivity), n(%)	349 (68)	18 (69)	331 (68)
Anti-CCP (positivity), n(%)	319 (62)	20 (77)	299 (62)
Disease duration at baseline (years), mean±SD	12.6±8.8	18.2±11.5	12.3±8.6
DAS28, mean±SD	4.1±1.4	3.9±1.4	4.1±1.4
Swollen joint count, median (IQR)	4 (1-7)	2 (0-7)	4 (1-7)
Tender joint count, median (IQR)	3 (1-8)	2 (1-6)	3 (1-8)
ESR, (IQR)	17 (9-32)	18 (10-36)	17 (9-31)
VAS, (IQR)	40 (20-60)	38 (20-60)	40 (20-60)
DAS28 >5.1, n(%)	113 (22)	4 (15)	109 (23)
CRP, median (IQR)	7 (3-16)	8 (3-18)	7 (3-16)
HAQ, median, mean±SD	1.3 (0.4-1.9)	1.2 (0.3-2.1)	1.3 (0.4-1.9)
SCORE original (%), median (P25-P75)	8.3 (2.7-25.6)	32 (14-64)	8 (3-24)
SCORE updated (%), median (P25-P75)	8.2 (2.7-19.9)	23 (12-43)	7 (2-19)

i. BMI; body mass index, TC; total cholesterol, HDL; high density lipoprotein, LDL; low density lipoprotein, CVD; cardiovascular disease, anti-CCP; anti-cyclic citrullinated peptide, DAS28; 28-joint disease activity score, ESR; erythrocyte sedimentation rate, VAS; visual analogue scale (patient global VAS), CRP; c-reactive protein, HAQ; health assessment questionnaire. Hypertension is defined as multiple measurements of elevated systolic blood pressure (>140 mmHg) during multiple visits by a physician. Diabetes mellitus includes both type I and type II. All variables represent baseline measures, except when otherwise stated.

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CHAPTER 9

GENERAL DISCUSSION

Photo by Volkan Olmez

The risk of developing CVD is notably higher in RA patients compared to the general population. Although a decrease has been reported in recent years, CVD mortality remains elevated in RA patients.[1] In order to reduce this risk, CVD risk management should be an integral part of clinical care for patients with RA. This would entail screening for CVD risk factors and identifying patients with a high risk of CVD. It was hypothesized that risk algorithms used in the general population may be less suitable to use for CVD risk assessment in RA as traditional risk factors do not fully account for the excess CVD risk in RA patients.[2, 3, 4, 5] In addition, certain RA specific factors may serve as a predictor, particularly chronic systemic inflammation.[6, 7] Systemic inflammation is a hallmark of RA, which has been a focal point of research investigating CVD risk in this population. Whilst there are various ways in which systemic inflammation is hypothesized to increase CVD risk, the exact mechanism underlying the association between inflammation and CVD in RA remains largely unknown. Evidence supporting the application of (disease specific) CVD risk management guidelines in RA is limited. It is unclear what strategy is most effective for accurate detection of RA patients at risk for developing CVD, and what predictors would be best suited for CVD risk estimation in RA. This information is of importance for appropriate allocation of preventative treatment, thus for the reduction of CVD morbidity and mortality in this population. Therefore, **the main focus of this thesis was on CVD risk prediction and on the CVD risk profile in RA patients**. The first objective was to evaluate the predictive performance of various CVD risk algorithms used in the general population and to develop and evaluate a risk algorithm that includes disease specific risk factors in RA patients. The second objective was to shed more light on the relationship between disease activity and the risk of developing CVD in these patients. In summary, the main conclusions of this thesis are as follows.

Chapter 2: A modest storage decay effect on lipoproteins determined in frozen serum samples was found that is unlikely to significantly affect CVD risk stratification. Serum samples that have been stored long-term (>10 years) can be used to obtain valid lipid measurements for developing CVD risk prediction models in RA cohorts, even without applying a decay correction factor.

Chapter 3: Risk estimates by traditional CVD risk algorithms, when applied in RA population, were found to be less accurate compared to reports from the general population. Overall, they largely underestimated CVD risk in RA patients, which may lead to insufficient application of preventive measures and therapies.

Chapter 4 & 5: The TC:HDL-c ratio, or atherogenic index (AI) and HDL-function may be more suitable parameters of the lipid profile to serve as CVD risk predictors in patients with RA than individual measurements of lipids. Based on the available literature, it appears that biological DMARDs are able to modulate the lipid profile in RA. Interestingly, these changes in individual lipid levels do not always translate into changes in the AI or are not sustained long enough to significantly affect this ratio. Inflammation may also diminish the beneficial effect of HDL-c by affecting its composition. This may additionally reduce the predictive value of the lipoprotein concentrations used in CVD risk assessment in RA. Perhaps these effects differ between men and women. To counteract these harmful effects of inflammation on the lipid profile, striving for tight control of disease activity may be of importance.

Chapter 6 & 7: Disease duration alone does not independently affect the risk of CVD and may therefore not be suitable as a disease-specific predictor when estimating CVD risk in individual RA patients. Disease activity on the other hand appears to augment CVD risk, particularly high uncontrolled disease activity over time. Conversely, low disease activity over time was found to be protective against CVD in this population. RA patients with a DAS28 over time ≤3.2 have a significantly lower risk of CVD than patients with active disease. DAS28-remission does not appear to significantly add to this beneficial effect. These findings further support the rationale of using tight control as a preventive strategy in CVD risk management in RA patients.

Chapter 8: Adaptations of the SCORE algorithm that include disease specific risk factors do not provide sufficient improvement in the predictive performance of this model and therefore it is not an appropriate alternative to the original SCORE. However, risk estimation using the original SCORE algorithm may underestimate CVD risk in RA patients.

Cardiovascular disease risk prediction in RA

A risk prediction algorithm is a useful tool for identifying individuals at significant risk for developing CVD and subsequently for allocating preventive treatment to those patients. Effective CVD risk management requires assessment of (modifiable) risk factors, and includes treatment and lifestyle changes that will significantly reduce CVD risk in individuals.[8] As alluded before, the traditional risk factors that are used for this purpose in the general population do not fully account for the excess risk that is reported in the RA population, even though some of the traditional risk factors appear to be more prevalent in RA patients.[2-5, 9-12, 13] We have demonstrated that the predictive performance of four currently available CVD risk models, which are based on traditional risk factors, is not satisfactory.[**Chapter 3**] Accordingly, the risk of future CVD is underestimated in a large group of RA patients, which may hamper adequate and timely preventive action in patients with intermediate and high risk of CVD. This finding is supported by the results of other studies in which the predictive performance of traditional CVD risk models in RA patients were evaluated in US and Southern European populations.[14-16] These findings raise questions concerning the role of currently available CVD risk algorithms in clinical decision making and allocation of preventive care in RA patients. In the 2011 EULAR recommendations for CVD risk management in RA it was suggested that in the presence of certain disease specific risk factors (disease duration >10 years, rheumatoid factor or anti-CCP positivity, presence of extra- articular manifestations) a 1.5 multiplication factor should be applied to the risk estimate calculated with a risk algorithm such as SCORE. Although these recommendations generated attention for CVD risk management in RA, the multiplication factor appears to be too crude a measure to significantly improve CVD risk predictions, as was demonstrated by us and others.[15, 17, 18] Also, disease duration, rheumatoid factor and anti-CCP positivity were found not to be significant predictors of CVD in our cohort. However, there is no suitable alternative at the moment that is proven to be more accurate or superior as a CVD risk prediction tool in RA. Therefore a recent update of the EULAR recommendations for CVD risk management in the RA population still advocates the use of this multiplication factor as the most evidence based CVD risk algorithm in RA patients, in addition to 9 other recommendations.[19] As existing prediction rules tend to underestimate CVD risk in a large portion of RA patients and the suggested multiplication factor

does not appear to reclassify sufficient RA patients to a more fitting risk category, there is a risk of under-treating modifiable traditional risk factors in RA patients when following these guidelines. Further insight into the relationship between disease specific risk factors and CVD may facilitate development of a more accurate risk prediction algorithm.

The role of systemic inflammation in the cardiovascular disease risk profile in RA

When it comes to the excess risk of CVD in RA and the search for potential RA specific CVD risk factors, there has been a particular interest in systemic inflammatory activity, which seems to be associated with the development of CVD.[2, 6, 7, 20, 21] The complexity of the mechanisms underlying the effect of systemic inflammation on CVD risk are still is not fully understood. Atherosclerosis has been described as an inflammatory disease,[7] which led to the hypothesis that chronic systemic inflammation may augment this process. Furthermore, inflammation may work synergistically with traditional risk factors to further augment CVD risk. Increased cytokine levels in RA have been associated with insulin resistance.[22] In combination with reduced physical activity levels this may lead to the development of metabolic syndrome; a collection of risk factors for the development of CVD.[23, 24] Furthermore, fluctuations in inflammatory activity or disease activity in RA patients appear to affect lipoproteins levels. The AI seems to be less affected and more stable, rendering it a more suitable determinant for CVD risk.[**Chapter 5**] Use of this ratio is therefore also advised in the EULAR recommendations for CVD risk management in RA.[19] Overall, lipoproteins appear to decrease under the influence of systemic inflammation. The traditional interpretation of decreased cholesterol levels would be that this is effect is therefore beneficial as it would reduce CVD risk. However, inflammation appears to have another effect on lipoproteins. In RA patients, the composition of HDL-c is altered [**Chapter 4**] and the anti- atherogenic function of HDL-c may be diminished, even become pro-atherogenic as a result of the effects of systemic inflammation.[25-28] Of note, these changes appear to be associated with an increased CVD event rate. A high apolipoprotein (apo)B/apoA1 ratio was found to be predictive of CVD events during 18 years of follow-up in 74 RA patients with active disease.[29] ApoA1 is an important protein constituent of HDL-c, involved in reversed cholesterol transport, contributing heavily to the anti-atherogenic properties of HDL-c. These findings could also have consequences for the predictive power of the AI, that includes HDL-c, when assessing CVD risk in patients with active disease [**Chapter 4, 5**] Conversely, anti-rheumatic therapies in responding RA patients can increase lipid levels, reflecting the suppression of inflammatory activity.[**Chapter 5**] In addition to an increase of HDL-c, the anti-atherogenic properties of HDL-c were found to be improved.[25,27, 30] The long-term effects of these changes on CVD morbidity and mortality have yet to be determined. Other traditional risk factors have been investigated in RA as well. Hypertension appears to be more prevalent in RA [31] and was a significant predictor for CVD in our cohort. Male gender and age are non-modifiable CVD risk factors in the general population. However, in a study by Fransen et al.[32] male and female patients with RA were shown to have similarly increased relative risks of CVD, which is interesting considering that male gender alone is regarded as an important risk factor for CVD in the general population. In this study, the youngest patients were also found to have the highest relative risk of developing CVD when compared to the oldest patients.[32] Another study demonstrated that male gender, as

well as smoking and cardiac history had weaker associations with CVD compared to a non-RA population.[4] These findings could indicate that in RA, the predictive power of certain traditional risk factors such as gender and age is different from the general population. Overall, inflammation appears to modify the effect of traditional CVD risk factors, effectively changing their relative contributions to CVD risk in RA. Therefore, the performance of CVD risk algorithms could benefit from recalibration of the respective weights of individual risk factors in these models.

In addition to interacting with traditional risk factors, inflammation may also be an independent risk factor for developing CVD. Overall, determining in precise terms the association between systemic inflammation and the development of actual CVD events has proven to be challenging. In part, this may be due to the fact that disease activity tends to fluctuate over time in most RA patients and the measurements reflect just moments of disease activity during follow-up. There are many other factors that may affect either disease activity and/or the risk of CVD, and could potentially act as confounders for this relationship. These methodological aspects are not always properly accounted for in the available literature on this topic. In the studies discussed in **Chapter 6** and **7** these factors were taken into account. Based on our research it appears that disease duration, or the time that disease activity exerts its influence, is not a strong independent predictor for future CVD in RA.[**Chapter 6**] Disease activity however does appear to have a significant effect on the risk of CVD which seems to be most apparent in patients who are more consistently on the higher or lower end of the disease activity spectrum. Illustratively, a high DAS28 at baseline and moderate to high, uncontrolled disease activity over time were found to significantly augment CVD risk.[**Chapter 6, 7**] We were able to demonstrate this effect in a large cohort of RA patients with prolonged follow-up. Recently, others have also demonstrated the unfavorable effect of a high cumulative burden of severe RA disease, and frequent flare-ups on CVD risk.[33, 34] Conversely, patients who are able to achieve very low disease activity over time have a significantly lower risk of CVD compared to patients with more active disease.[**Chapter 7**] Of note, remission does not appear to significantly add to this beneficial effect. Achieving low, stable disease activity (DAS28≤3.2) may be a significant step towards substantially reducing CVD risk in RA.

Overall, a considerable body of evidence supports the notion that systemic inflammation has an important role in the development of CVD in RA. In concurrence with these findings, the results discussed in this thesis indicate a significant association of disease activity with occurrence of CVD in RA although this is most apparent in patients with sustained high or very low disease activity over time. Our findings underline the importance of tight control as a strategy in CVD risk management in the RA population, now also propagated by several other research groups.[1, 19, 35] A subgroup of RA patients with severe, poorly controlled disease may have the most to gain from adequate CVD risk management.

Cardiovascular disease risk management in RA

The evidence supporting the role of systemic inflammation as both an independent CVD risk factor and a modulator of traditional risk factors has led to the 'smaller slice of a large pie'-concept

(see figure 1.1, in the introduction).[36] This hypothesis states that the overall risk of CVD is higher in RA and although traditional risk factors do contribute to the CVD risk profile of RA patients, their relative contribution to CVD risk is smaller compared to the general population. As discussed before, the CVD risk profile of those RA patients who have a high risk of developing CVD may be significantly different from the risk profile in the general population. Simply adjusting or recalibrating an existing CVD risk algorithm does not seem to be sufficient as it does not result in a significant improvement in the accuracy of 10-year CVD risk estimates [**Chapter 8**]. The risk estimates calculated with the adjusted SCORE algorithm did correct some of the underestimation of CVD risk, also including a portion of RA patients who would otherwise be categorized as low or intermediate CVD risk and who would therefore be unlikely to receive adequate preventive therapy. Unfortunately, the predictive performance of the adjusted SCORE algorithm did not significantly improve compared to the original SCORE.[**Chapter 8**] A more radical approach would be to build a prediction model from scratch without including traditional risk factors 'by default'. This could perhaps generate a more powerful and accurate CVD prediction algorithm. Several other CVD-related parameters that have been discussed elsewhere, such as carotid artery intima-media thickness and presence and composition of atherosclerotic carotid plaques could contribute to more accurate CVD risk prediction. Screening for asymptomatic atherosclerotic plaques by means of carotid ultrasound has been recommended as part of CVD risk assessment in RA patients.[19] Although these measurements may have potential as predictors used to identify RA patients who are most at risk for developing CVD, several other aspects play a role when determining what CVD risk factors are suitable for use in clinical practice.[37, 38, 39, 40] The practicality and cost-benefit ratio of measuring and monitoring certain disease specific risk factors in clinical practice should also be considered. On a different note, there is no evidence to suggest that treatment strategies or lifestyle changes aimed at reducing traditional risk factors would be ineffective. In the 2015/2016 update of the EULAR recommendations for CVD risk management the authors point out that statins appear to be equally effective in RA patients.[19] Results from the Trial of Atorvastatin for the primary prevention of Cardiovascular Events in patients with Rheumatoid Arthritis (TRACE- RA) that included 2986 RA patients with 7908 patient years of follow-up show that statins are safe and effective for use in RA patients. A significant reduction in LDL-c levels was achieved in patients randomized to 40mg of atorvastatin daily, compared to placebo. Also, a 34% reduction in CVD events was reported in the intervention group, although this difference did not reach statistical significance.[41] Smoking cessation, a healthy diet and exercise is recommended as a part of RA management for beneficial effects on both CVD risk and disease outcomes.[19] Even though traditional risk factors do not fully explain the excess CVD risk, they should not be considered irrelevant. At this point, there is no reason to withhold appropriate preventive measures targeting traditional CVD risk factors in patients with RA.

Conclusion and future recommendations

RA patients have an increased risk of CVD, and systemic inflammation is likely to be a crucial contributor herein, both as an independent risk factor and by modulating other traditional CVD risk factors. Currently available CVD risk algorithms provide suboptimal risk estimates in these

patients, which may lead to ineffective allocation of preventive measures. A disease specific CVD risk algorithm, tailored to the CVD risk profile of RA patients, may provide a solution to this problem although a simple adjustment of existing risk algorithms does not seem sufficient. In addition to the individual predictive ability of traditional risk factors and disease activity, the relative contribution of each risk factor should be carefully evaluated when developing a disease specific CVD risk algorithm. Until an improved alternative is available, using existing guidelines and recommendations may be the best option for CVD risk assessment and management in RA patients. Creating awareness of the increased CVD risk and of the drawbacks of using existing risk algorithms and guidelines in the RA population among healthcare providers may contribute to improved allocation of preventive care. Additionally, as disease activity appears to be an important addition to the CVD risk profile of RA patients, striving for tight control of disease activity during the course of RA is of importance, not only to prevent joint damage but also to reduce the risk of CVD. Particularly patients with severe disease activity at diagnosis and/or those who are inadequately responding to anti-rheumatic therapy over time may require stringent monitoring of disease activity and traditional risk factors whilst maintaining low thresholds for initiating preventive care to effectively prevent future CVD. Additional research is necessary to investigate the added value of more tailored, disease specific strategies to assess and reduce CVD risk in patients with RA. This means further elucidating the mechanisms underlying the effect of systemic inflammation on CVD risk, investigating the long-term effects of tight-control on CVD risk and to continue to explore the potential of disease specific CVD risk factors that may facilitate the identification of high risk patients.

Lastly, this thesis hopefully encourages rheumatologists individually to take steps to embed cardiovascular risk management in the routine care of their RA patients.

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9

CHAPTER 10

SUMMARY

Patients with rheumatoid arthritis (RA) have an increased risk of cardiovascular disease (CVD) compared to the general population. In order to reduce the risk of CVD, cardiovascular risk management should be an integral part of (clinical) care for patients with RA. **The main focus of this thesis was on CVD risk prediction in RA and on the CVD risk profile in RA patients.** The first objective was to evaluate in RA patients the predictive performance of various CVD risk algorithms that are used in the general population to predict the 10-year risk of CVD. Additionally, a CVD risk algorithm that includes disease specific risk factors was developed and evaluated. The second objective was to investigate the role of inflammation in the CVD risk profile of RA patients and to particularly shed more light on the relationship between disease activity and the risk of developing CVD. For the research discussed in this thesis, the 1985 early RA inception cohort was used as the main source of data. Cholesterol levels were not measured systematically, as is the case in many large RA cohorts. Therefore, the first step was to validate the use of frozen serum samples. As described in **Chapter 2**, only a modest storage decay effect on lipoproteins was found that was unlikely to significantly affect CVD risk stratification. It was demonstrated that even after long time storage frozen serum samples are a valid source to obtain lipid measurements for CVD oriented research in RA. Next the performance of four traditional CVD risk algorithms was evaluated in the RA cohort in **Chapter 3**. These four algorithms proved to be less accurate in RA patients compared to results from the general population. Further insight into the relationship between disease specific risk factors and CVD may facilitate development of a more accurate risk prediction algorithm. There has been a particular interest in systemic inflammatory activity, as it seems to be associated with the development of CVD. In **Chapter 4** the effect of inflammation on lipids was investigated by comparing the composition of HDL-c in RA patients to healthy controls. Inflammation appears to change the composition of HDL-c, which may contribute to diminished anti-atherogenic properties of HDL-c in RA patients. Altered function of HDL-c as a result of inflammatory activity was also found when reviewing the literature on the effect of anti-inflammatory therapy on lipoproteins in **Chapter 5**. Furthermore, results showed that biological DMARDs appear to be able to modulate the lipid profile in RA whilst these changes often do not translate into changes in the relative TC:HDL-c ratio or atherogenic index (AI). Therefore, AI and HDL-function may be more suitable parameters of the lipid profile as determinants of CV risk in patients with RA. In addition to an interaction with traditional risk factors, systemic inflammation may also be an independent risk factor for developing CVD. In **Chapter 6** this hypothesis was explored by investigating the effect of both disease duration and disease activity on the risk of CVD in RA. Disease duration alone does not significantly affect the risk of CVD. Disease activity over time on the other hand appears to augment CVD risk, particularly high uncontrolled disease activity over time. Furthermore, as demonstrated in **Chapter 7,** it was found that low disease activity over time (DAS28≤3.2) is protective against the development of CVD. Of note, remission does not appear to significantly add to this beneficial effect. These results further support the rationale of using tight control as a preventive strategy in CVD risk management in RA patients. Furthermore, it appears that the risk profile of RA patients who are at high risk for developing CVD is significantly different from that of the general population. This also affects CVD risk prediction. Unfortunately, as was shown in **Chapter 8,** simply adjusting or recalibrating an existing CVD risk algorithm does not seem to be sufficient as it does not result in a significant improvement

in the accuracy of CVD risk estimates. A more rigorous approach for developing a CVD risk algorithm, that also incorporates re-evaluation of the individual contributions of all traditional CVD risk factors to the RA specific CVD risk profile, may yield more promising results.

In conclusion, RA patients have an increased risk of CVD and systemic inflammation is likely to be a crucial contributor herein. Inflammation appears to act as an independent risk factor and also by modulating other traditional CVD risk factors. Currently available CVD risk algorithms provide suboptimal risk estimates in RA patients. However, as there is no suitable alternative available at this time, using existing guidelines and recommendations may be the best option for CVD risk assessment and management whilst keeping in mind the drawbacks of using these tools in the RA population. In addition to monitoring and treatment of traditional CVD risk factors, striving for tight control of disease activity during the course of RA is of importance in this regard.

CHAPTER 11

SAMENVATTING

Patiënten met reumatoïde artritis (RA) hebben een verhoogd risico op cardiovasculaire ziekten ofwel hart- en vaatziekten (HVZ) in vergelijking met de algemene populatie. Om dit risico te verminderen zou cardiovasculair risicomanagement een integraal onderdeel moeten vormen van de zorg voor patiënten met RA. **In dit proefschrift is gefocust op de predictie van het individuele risico op hart- en vaatziekten bij RA patiënten en op het verder definiëren van het cardiovasculair risicoprofiel van patiënten met RA.** Eerst is daarom de toepassing van verschillende bestaande cardiovasculaire risicomodellen in de RA-populatie geëvalueerd en is er een bestaand risicomodel aangepast door RA-specifieke risicofactoren voor het ontwikkelen van HVZ te includeren in het model. Daarnaast is de rol van ontstekingsactiviteit in het cardiovasculaire risicoprofiel van RA-patiënten onderzocht waarbij specifiek is gekeken naar de relatie tussen gemeten ziekteactiviteit en het risico op hart- en vaatziekten bij patiënten met RA. Voor het verrichte onderzoek dat besproken wordt in dit proefschrift is het Nijmeegse vroege RA inceptiecohort gebruikt als de belangrijkste bron van data. In dit cohort werden cholesterolmetingen niet routinematig verricht, wat het geval is in veel grote RA-cohorten. Daarom bestond de allereerste stap uit het valideren van het gebruik van bevroren serummonsters, die wel voorhanden waren voor deze patiënten, voor de cholesterolmetingen. Zoals beschreven in **Hoofdstuk 2**, werd er als gevolg van het verval van lipoproteïnepartikels in het serum tijdens langdurige opslag een bescheiden effect geobserveerd op de hoogte van de gemeten lipoproteïne waardes. Het is onwaarschijnlijk dat dit een significant effect heeft op schattingen van het cardiovasculair risico. Als volgende stap werden vier bestaande cardiovasculaire risicomodellen, veel gebruikt in de algemene populatie, in een RA-cohort geëvalueerd. Dit is besproken in **Hoofdstuk 3**. Deze vier modellen bleken duidelijk minder accurate schattingen van het cardiovasculair risico te geven in de groep RA-patiënten in vergelijking met behaalde resultaten in de algemene populatie. Resultaten lieten zien dat het cardiovasculair risico in een grote groep RA-patiënten werd onderschat. Dieper inzicht in de relatie tussen ziekte specifieke risicofactoren en HVZ kan het ontwikkelen van een accurater predictiemodel gericht op de RA-patiënt verder faciliteren. In dat opzicht is het met name interessant te kijken naar systemische ontstekingsactiviteit, wat geassocieerd wordt met de ontwikkeling van HVZ bij patiënten met RA. In **Hoofdstuk 4** wordt de studie besproken waarin het effect van inflammatie op lipiden is onderzocht door de samenstelling van het als gunstig bekend staande high-density lipoprotein cholesterol (HDL-c) bij RA-patiënten te vergelijken met gezonde controles. De compositie van HDL-c partikels lijkt onder invloed van ontstekingsactiviteit te veranderen, wat kan bijdragen aan de verminderde anti-atherogene, gunstige, functie van HDL-c. Een veranderde functie van HDL-c werd ook gerapporteerd door andere studies die zijn besproken in de systematische literatuur review in **Hoofdstuk 5.** Daarnaast lieten resultaten gepresenteerd in dit hoofdstuk ook zien dat disease modifying anti-rheumatic drugs (DMARDs) die ontstekingsactiviteit onderdrukken invloed lijken te hebben op het lipidenprofiel bij RA-patiënten. Deze veranderingen leiden echter meestal niet tot significante veranderingen in de relatieve TC:HDL-c ratio, ofwel atherogenic index (AI). Gezien deze bevindingen lijken deze TC:HDL-c ratio en HDL-c functie meer geschikte parameters van het lipidenprofiel om te includeren in het cardiovasculair risicoprofiel van RA-patiënten. Naast een interactie tussen ontstekingsactiviteit en traditionele risicofactoren lijkt ontsteking ook als een onafhankelijke risicofactor te werken. In **Hoofdstuk 6** werd dit verder onderzocht door het effect van zowel

ziekteactiviteit als ziekteduur op het risico op HVZ bij RA-patiënten te analyseren. Ziekteduur alleen lijkt geen significante onafhankelijke risicofactor te zijn in dat opzicht. Hoge ongecontroleerde ziekteactiviteit daarentegen lijkt onafhankelijk het risico op HVZ significant te verhogen. Verder werd gevonden dat lage ziekteactiviteit beschermend werkt tegen de ontwikkeling van HVZ, zoals behandeld in Hoofdstuk 7. Opvallend genoeg bleek uit de besproken resultaten dat remissie gemeten met de DAS28 (score voor ziekteactiviteit) geen significante toevoeging lijkt te leveren aan dit gunstige effect. Deze bevindingen ondersteunen de motivering voor direct strakke regulering of 'tight control' van ziekteactiviteit bij patiënten met RA als preventieve strategie voor het voorkomen van HVZ in deze populatie. Daarnaast lijkt het cardiovasculair risicoprofiel van patiënten met RA aanzienlijk te verschillen van het risicoprofiel in de algemene populatie. Dit beïnvloed daarmee ook de risicoschatting met behulp van risicomodellen die gebaseerd zijn op enkel traditionele risicofactoren uit de algemene populatie. Helaas lijkt een eenvoudige aanpassing van een bestaand risicomodel niet voldoende voor een significante verbetering van individuele schattingen van het cardiovasculair risico bij patiënten met RA.[Hoofstuk 8]

Concluderend hebben patiënten met RA een verhoogd risico op HVZ waarbij systemische ontstekingsactiviteit een centrale rol lijkt te spelen; zowel als een onafhankelijke risicofactor, als door effectmodulatie van andere traditionele risicofactoren. Het risicoprofiel van RA-patiënten lijkt daarmee te opvallend te verschillen van de algemene populatie. De beschikbare cardiovasculaire risicomodellen leveren suboptimale risicoschattingen bij RA-patiënten. Er is echter geen geschikt alternatief beschikbaar op dit moment. Daarom is het gebruik van bestaande richtlijnen en aanbevelingen op het gebied van cardiovasculair risicomanagement mogelijk voor nu de beste oplossing, waarbij men bovengenoemde resultaten in het achterhoofd houdt wanneer deze toegepast worden op patiënten met RA. Naast aandacht voor traditionele risicofactoren is het nastreven van een strakke regulatie van ziekteactiviteit gedurende het beloop van RA daarbij belangrijk.

DANKWOORD

Dit hoofdstuk zal voor de meeste lezers het eerste (en soms het enige) hoofdstuk zijn dat goed bekeken wordt. Het is in mijn beleving dan ook een erg belangrijk hoofdstuk. Dit proefschrift dient als een proeve van bekwaamheid tot het zelfstandig beoefenen van de wetenschap. In mijn geval levert het ook het bewijs voor de inzet, steun en hulp van een heel fijne groep mensen waarvan ik mezelf gelukkig een deelgenoot mag noemen. Graag wil ik jullie hiervoor bedanken.

Als eerste Prof. van Riel, Piet, mijn promotor. Je hebt me aangenomen vers uit de schoolbanken en me de kans gegeven om mijn project mede vorm te geven, vertrouwen te krijgen en mezelf te ontwikkelen. Toen er zich de mogelijkheid voordeed om met mijn onderzoek onderdeel te gaan uitmaken van een internationaal samenwerkingsverband heb jij je daar enorm voor ingezet en gezorgd dat er voor mij een rol was weggelegd. Toen ik met je besprak dat ik weer wilde gaan studeren heb je me hierin gesteund en geholpen dit mogelijk te maken voor mij. Ik heb zelfs nog een tijdje bij je in dienst mogen werken als onderzoeker toen er elders een leuk project lag. Ik kwam graag bij je langs voor advies of om even bij te praten. Bedankt!

Jaap, als copromotor heb je mij al die jaren bijgestaan met gedegen advies, hulp en een luisterend oor. Je was zelf vaak een man van weinig woorden, en de woorden die gesproken werden volgden elkaar in bijna onnavolgbare snelheid op. Ik vind het hier op papier nu wel veilig om toe te geven dat de eerste maand van mijn aanstelling ik vaak op goed geluk interpreteerde wat je tegen me zei. Altijd kon ik bij je terecht met zorgen over het onderzoek. Jouw rust en bedachtzaamheid gaven mij het vertrouwen dat geen enkele crisis onoverkoombaar was. Ik dumpte mijn stress als het ware op jouw bureau en kon dan weer met een helder hoofd jouw werkkamer verlaten. Zelfs mijn vaardigheden op het gebied van methodologie en statistiek heb ik dankzij jouw begeleiding uit het slop weten te trekken. Het stelt me nu in staat mijn eigen werk, maar ook dat van anderen kritisch te beoordelen en op waarde te schatten. Dit maakt me een beter onderzoeker en een betere dokter. Bedankt dat je altijd kritische vragen stelde, dat je eindeloos geduld met me had als ik zelf met duizend vragen kwam of het niet direct begreep. En bedankt voor de vreugde die ik bij je bespeurde als ik zelf een probleem had geconstateerd en opgelost, dat was een belangrijke motivator.

Calin, nadat ik jouw proefschrift aan het begin van mijn promotietraject onder ogen kreeg was ik hiervan behoorlijk onder de indruk en werd de lat meteen een paar verdiepingen hoger gelegd. Jouw inbreng en feedback op mijn stukken was altijd scherp en zorgde ervoor dat ik een beter schrijver werd. Bedankt voor je fijne begeleiding als copromotor. Je hebt bij mij het enthousiasme voor het onderwerp van mijn thesis verder aangewakkerd en ervoor gezorgd dat er een duidelijke lijn kwam in mijn ideeën. Samen hebben we ook gewerkt aan een cardiovasculaire screening op de poli reumatische ziekten. Hoewel ik soms het idee had dat je mijn felheid of mijn manier van doen wel vermakelijk vond voelde ik me altijd serieus genomen en op waarde geschat. Bedankt.

Rogier, menig ingewikkeld statistisch vraagstuk heb ik bij jou neergelegd en hoe druk je ook was, altijd kwam er een oplossing. Bedankt daarvoor.

En dan mijn Reuma-collega's. Bea, met jou deelde ik als eerste een kamer en mijn onderzoek borduurde deels voort op het werk dat jij al had verricht. Bedankt dat je me op sleeptouw nam. Franka en Sjoukje, bedankt voor jullie gezelligheid en de interesse die jullie altijd hebben getoond in de (idiote) dingen die ik allemaal bedenk. Han, bedankt voor de fijne samenwerking. Je bood me een fris perspectief en jouw rust en genuanceerde kijk op de zaken heb ik altijd erg kunnen

waarderen. Wietske, jij was niet officieel mijn begeleider maar zo heb ik dat stiekem wel altijd een beetje ervaren. Een sterke ambitieuze vrouw ontbrak namelijk nog aan dat rijtje. Ik kon het altijd goed met je vinden en soms enorm met je lachen. Zo denk ik nog weleens terug aan de avond dat we aan een krakkemikkig tafeltje op het terras van een piepklein restaurantje ergens verscholen in Rome zo'n anderhalf uur de slappe lach hebben gehad. Geen idee meer waar het over ging maar wel een heel fijne herinnering aan mijn eerste congres. Bedankt!

Jos, Kavish, Sanne, Sofie, Yvonne, Marieke en Wieneke. Met jullie heb ik deels in de barakken en deels op 'de -2' mogen bivakkeren, beide onvergetelijke werkplekken. Naast dat ik aan jullie fijne sparringpartners heb gehad om te praten over het onderzoek en alles wat daarbij komt kijken, kon ik ook bij jullie terecht als de frustraties hoog opliepen en heb ik aan jullie allemaal heel fijne, gezellige collega's gehad. Bedankt! En wat een hoop onzin is er uitgekraamd en uitgespookt, met name door het illustere duo Hendrikx en Bhansing, tot veel vermaak van mij en jullie kamergenoten. Woorden die omhoog borrelen zijn, rotjes, slingers en feestmutsen, mandarijnen en 'practical jokes'. Dankzij jullie heb ik bij 'wie is de mol' nu naast het voelen van intense afkeer ook een aantal positieve associaties. Wat erg fijn is omdat dit programma waarschijnlijk nog 25 seizoenen lang uitgezonden gaat worden. Wieneke en Kavish, bij jullie kon ik altijd terecht met vragen over de opleiding geneeskunde en alle keuzes en overwegingen over het traject daarna, wat mijn episodische opvlammingen van toekomststress absoluut ten goede is gekomen. Jos, je bent er als enige verantwoordelijk voor dat ik ooit begonnen ben aan het zelf schrijven van SPSS-syntax. Dat gebruik ik nu nog om indruk te maken op feestjes. Ook voor vragen over de beste breedhoeklens, filters, computerprogramma's, tips voor het zelf regelen van reizen naar verre bestemmingen of wanneer ik enthousiastelingen nodig had voor een roadtrip kon ik bij jou terecht. Dankjewel! Sofie, jouw gevoel voor humor, no-nonsense houding en gematigde blijheid op de maandagochtend heb ik altijd erg gewaardeerd en ik kon met jou ook heerlijk over andere bezigheden dan onderzoek ouwehoeren. Bedankt! Yvonne, het feit dat je in mijn ogen altijd je eigen plan hebt getrokken bewonder ik aan jou. Bedankt voor de vele fijne gesprekken die ik met jou heb gehad, ik voelde me altijd erg gehoord. Marieke en Sanne, bedankt voor jullie collegialiteit, gezelligheid en nuchtere kijk op de wereld. Graag wil ik ook nog iedereen van het datacentrum, het secretariaat en alle andere voormalig collega's van de afdeling reumatische ziekten bedanken voor de fijne samenwerking en hulp die ik heb mogen ontvangen!

Eugene, ik kwam nogal last-minute bij je met het verzoek een ontwerp voor mijn kaft te maken. Ik stak hierbij een warrig verhaal af over mijn sterke gevoelens jegens primaire kleurcombinaties, cliché symbolen en stock-foto's, eigenlijk alles wat ik níét wilde. Op de een of andere manier heb je daar precies uit opgemaakt wat ik mooi vind en wat bij mij past. Enorm bedankt hiervoor.

Cogroep 178; Anissa, Dominique, Claudia, Eva, Jiska, Karlijn, Kelly, Koen, Lars, Laura, Leontien, Liset, Marc, Melanie, Milad, Mirthe, Moniek, Nadine, Roel, Stefanie, Vivianne, Wout, Mehmed, Rowie, Ngoc Lan, Daan en Bart (ik hoop dat ik niemand vergeet!). Halverwege mijn promotietraject kwamen jullie op mijn pad. Wat een mooie, gekke ervaring hebben we met elkaar gedeeld en wat fijn dat jullie deze oude bok gewoon opnamen in de groep. Dankzij jullie is mijn geestelijke veroudering wat afgeremd en heb ik mijn studententijd nog ongegeneerd een aantal jaar mogen rekken. Moniek, over een paar jaar kan ik het dankwoord in jouw boekje te lezen!

Laura, bedankt voor je enthousiasme en levenslust, dat werkt aanstekelijk. Stefanie bedankt voor al die keren dat je met een blik al aan het lachen kon maken, voor je gezelligheid en je eigenheid. Marc, bedankt voor je scherpe en geraffineerde gevoel voor humor, voor al die keren dat je me uitdaagt, bedankt dat ik zo lekker met je kan discussiëren en dat je mijn dokters-zielenmattie bent.

Aan iedereen thuis, bedankt voor alle keren dat we elkaar troffen in de kroeg/aan tafel/langs de lijn/tijdens het aflopen van de drukke sociale dorpskalender, en jullie interesse toonden of juist afleiding boden.

Mijn paranimfen, Miranda en Carli, bedankt dat jullie 'ja' hebben gezegd! Miranda, we kennen elkaar al vanaf de puberjaren en met jou heb ik een aantal van de mooiste ervaringen van mijn leven tot nu toe mogen beleven. Ook al zien we elkaar niet meer zoveel als vroeger, ik hoop je nog tot we oud en grijs zijn in mijn vizier te houden. Carli, ook van jou kan ik me niet eens meer goed herinneren dat je er niet was en in al die tijd is er denk ik geen onderwerp onbesproken gebleven. Je geeft me het gevoel dat ik altijd bij je kan aankloppen, en je humor, steun en weloverwogen advies waardeer ik enorm. Bedankt voor al onze gesprekken, voor je gezelligheid en alle dagjes weg de afgelopen jaren!

Cheryl, Vera en Daphne; ooit vriendinnen van langs de lijn en inmiddels vriendinnen voor het leven wat mij betreft. Jullie zijn een inspiratiebron voor mij op alle fronten. Bedankt voor jullie niet aflatende enthousiasme, inspiratie, interesse, attentie en niet te vergeten alle dansjes en wijntjes die we inmiddels gedeeld hebben. Ik voel me rijk in jullie midden. Wat zijn jullie prachtmensen!

Wiep, bij zo ongeveer elke mijlpaal in mijn leven (groot en klein) was je daar, camera in de aanslag om het allemaal vast te leggen. Bedankt dat je er bent, voor je belangstelling en voor het verzamelen van genoeg beeldmateriaal om bij mijn eerstvolgende verjaardag iedereen te kunnen trakteren op een avondvullende PowerPointpresentatie van mijn prestaties.

Ria, mijn surrogaat oma en eerste brenger van girlpower in mijn leven, bedankt.

Hetty, Geurt, Jozef, Guus, Janneke en Saskia, bedankt voor jullie steun, gezelligheid en interesse in mijn bezigheden. Mirthe en Sven, heerlijke moppies, iedere zaterdag is het voor mij weer een feest overladen te worden met zoveel liefde.

Kleine broertjes die inmiddels geen kleine broertjes meer zijn. Stef, relaxt met een hoofdletter R, niet drukker maken dan nodig is, en stoïcijns vertrouwen hebben in die levensvisie, daarin ben jij mijn voorbeeld. Derk, wellicht de intelligentste van ons drie en in ieder geval de denker, de filosoof en zeker de grootste en de luidste. Je houdt me scherp en zorgt tijdens familieactiviteiten altijd voor kwaliteitsentertainment. Bedankt bruurkes.

Lieve pap en mam, het feit dat dit boekje er ligt heb ik in de eerste plaats aan jullie te danken. De basis is door jullie gelegd. Pap, ik herken veel van de trekjes en karaktereigenschappen die ik waardeer aan mezelf in jou. Jij hebt me meer dan wie ook laten zien wat het waard is om iets te mogen doen waar je plezier in hebt en waar je voldoening uit haalt. Mam, mijn ambitie, doorzettingsvermogen, zelfvertrouwen en liefde voor de zorg heb ik vooral aan jou te danken. Bedankt voor jullie onvoorwaardelijke steun en voor het vertrouwen in dat jullie in mij hebben.

Lieve Daan, inmiddels al langer met jou dan zonder jou. Ik wil me mijn leven niet meer voorstellen zonder. Bedankt dat ik altijd op je kan terugvallen, dat je zoveel geduld met me hebt gehad de afgelopen jaren, dat je me zoveel liefde geeft en hier nooit aan lijkt te twijfelen, dat je altijd zonder blikken of blozen een stap terug doet om mij en mijn wilde plannen voor te laten gaan. We zijn zo ongeveer elkaars tegenpolen en dat maakt dat het nooit saai is met jou. Zonder jou was ik niet zover gekomen. Mijn dank is het grootst.

Over de auteur

Elke Arts, auteur van dit proefschrift, werd geboren op 26 juni 1986 in het Radboud UMC te Nijmegen. Zij behaalde in 2004 haar vwo-diploma, met het profiel 'economie en maatschappij' en biologie en Frans in het vrije deel, aan het Merlet College in Cuijk. Hierna startte zij met de opleiding Gezondheidswetenschappen aan de Universiteit van Maastricht waar zij zich specialiseerde in zorgwetenschappen en bewegingswetenschappen. Tijdens deze periode studeerde ze drie maanden in Zweden aan Mälardalen University in Västeras. In 2008 behaalde ze haar diploma voor de master Health Services Innovation waarvan de afstudeerscriptie getiteld "*Transferring diabetes care from the physician tot he diabetes nurse by means of substitution*" tot een publicatie geleid heeft, onder begeleiding van Prof. B. Vrijhoef en Prof. N. Schaper. Na een aantal maanden gereisd te hebben in Australië en Zuidoost-Azië vervolgde ze in 2009 haar wetenschappelijke carrière met een promotietraject op de afdeling Reumatische ziekten van het Radboud UMC te Nijmegen onder leiding van Prof. P. Van Riel, Dr. J. Fransen en Dr. C. Popa. Ze heeft hier onderzoek verricht naar het risico op hart- en vaatziekten bij patiënten met reumatoïde artritis, in het bijzonder naar risicomodellen en de rol van systemische ontstekingsactiviteit. Op verschillende nationale en internationale congressen heeft ze de resultaten van dit onderzoek mogen presenteren. Dit werk heeft in 2016 geresulteerd in het proefschrift dat voor u ligt. Tijdens haar promotietraject is ze in 2011 begonnen met de studie geneeskunde aan de Radboud Universiteit te Nijmegen. Haar masterdiploma behaalde ze in 2016. Momenteel is ze werkzaam als art-assistent op de spoedeisende hulp in het Canisius Wilhelmina Ziekenhuis te Nijmegen en verricht ze onderzoekstaken voor de afdeling kinderchirurgie van het RadboudUMC te Nijmegen.

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